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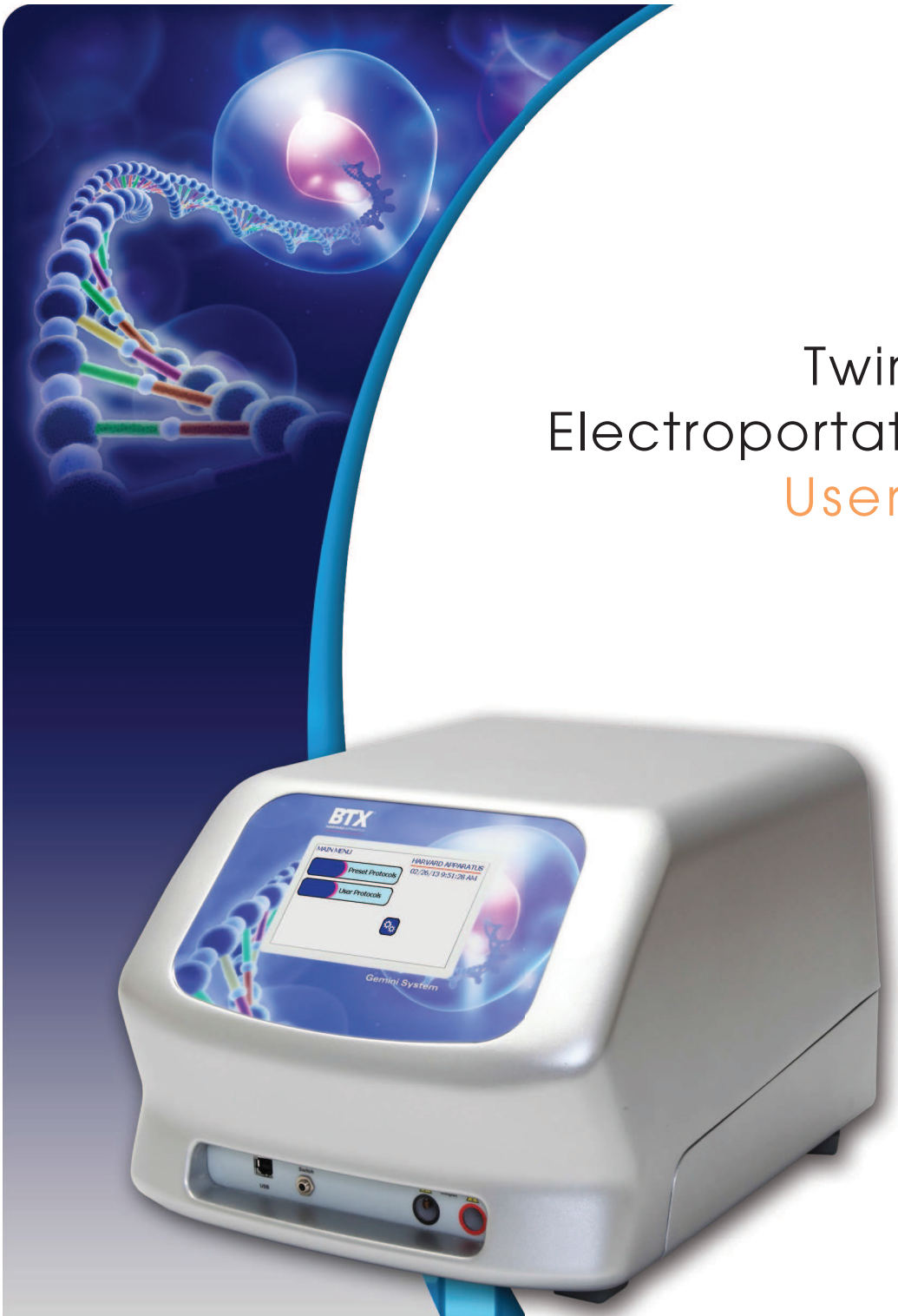
Gemini

Twin Waveform Electroportation Systems

Users's Manual



HARVARD APPARATUS
BTX®

www.btxonline.com

5507-002 Rev. A

Gemini Twin Waveform Electroportation Systems

RESEARCH ONLY

Harvard Apparatus BTX
84 October Hill Rd
Holliston, MA 01746, USA

Phone: 1-508-893-8999
Fax: 1-800-429-5732
Web: www.btxonline.com

Warranty

Harvard Apparatus warranties the BTX Gemini X² & BTX Gemini SC² Twin Waveform Electroporation Systems for a period of two years from the date of purchase. At its option, Harvard Apparatus BTX will repair or replace the unit if it is found to be defective as to workmanship or materials. This warranty does not extend to any instrumentation which has been (a) subjected to misuse, neglect, accident or abuse, (b) repaired or altered by anyone other than HARVARD APPARATUS BTX without HARVARD APPARATUS BTX express and prior approval, (c) used in violation of instructions furnished by HARVARD APPARATUS BTX. This warranty extends only to the original customer purchaser. IN NO EVENT SHALL HARVARD APPARATUS BTX BE LIABLE FOR INCIDENTAL OR CONSEQUENTIAL DAMAGES. Some states do not allow exclusion or limitation of incidental or consequential damages so the above limitation or exclusion may not apply to you. THERE ARE NO IMPLIED WARRANTIES OF MERCHANTABILITY, OR FITNESS FOR A PARTICULAR USE, OR OF ANY OTHER NATURE. Some states do not allow this limitation on an implied warranty, so the above limitation may not apply to you. Without limiting the generality of the foregoing, HARVARD APPARATUS BTX shall not be liable for any claims of any kind whatsoever, as to the equipment delivered or for non-delivery of equipment, and whether or not based on negligence. Warranty is void if the BTX Gemini X² & BTX Gemini SC² instrument is changed in any way from its original factory design or if repairs are attempted without written authorization by HARVARD APPARATUS BTX. Warranty is void if parts, connections or electrodes not manufactured by HARVARD APPARATUS BTX are used with the BTX Gemini X² & BTX Gemini SC² instrument. If a defect arises within the warranty period, promptly contact Harvard Apparatus BTX, 84 October Hill Road, Building 7, Holliston, Massachusetts, USA 01746-1388 using our toll free number 1-800-272-2775 (US Only) or 508-893-8999 (E-mail: techsupport.btx@harvardapparatus.com). Goods will not be accepted for return unless an RMA (Returned Materials Authorization) number has been issued by our customer service department. The customer is responsible for shipping charges. Please allow a reasonable period of time for completion of repairs, replacement and return. If the unit is replaced, the replacement unit is covered only for the remainder of the original warranty period dating from the purchase of the original device. This warranty gives you specific rights, and you may also have other rights, which vary from state to state.

These instruments contain a high voltage power supply adjustable to 3,000 V. High voltage power supplies present a serious risk of personal injury if not used in accordance with design and/or use specifications, if used in applications on products for which they are not intended or designed, or if they are used by untrained or unqualified personnel.

- The user must read this manual carefully before the instruments are placed into operation.
- Removing the cover will void the warranty.
- Do not connect or disconnect the high voltage cable with the high voltage enabled.
- To connect or disconnect the cable, turn line power off and unplug line (mains) cord.
- Do not touch the electrode tip while the waveforms are being applied

If there are any questions about the operation of this instrument, call Harvard Apparatus BTX Customer service at 1-800-272-2775, or 1-508-893-8999.

Out of Warranty Service

Proceed exactly as for Warranty Service above. If our service department can assist you by phone or other correspondence, we will be glad to help at no charge.

Repair service will be billed on the basis of labor and materials. A complete statement of time spent and materials used will be supplied. Shipment to Harvard Apparatus BTX should be prepaid. Your bill will include return shipment freight charges.

Disassembly by the user is prohibited. Service should only be carried out by experienced Harvard Apparatus BTX technicians.

Repair Facilities and Parts

Harvard Apparatus BTX stocks replacement and repair parts. When ordering, please describe parts as completely as possible, preferably using our part numbers. If practical, enclose a sample photo or drawing.

Caution Notice

The BTX Gemini X² & BTX Gemini SC² systems are intended for laboratory use only and can be used in research and development applications. These systems have been designed to meet the standards for electromagnetic compatibility (EMC) intended for laboratory equipment applications.

The BTX Gemini X² & BTX Gemini SC² systems comply with the applicable sections of IEC 61010-1:2012 3rd Ed – Safety requirements for electrical equipment for measurement, control, and laboratory use. This product should not be used in the presence of a flammable atmosphere such as an anesthetic mixture with air, oxygen, or nitrous oxide.

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Safety Information

Please read the following safety precautions to ensure proper use of your generator. If the equipment is used in a manner not specified, the protection provided by the equipment may be impaired.

Caution

Protective Ground Terminal

Caution Risk of Electric Shock

To Prevent Hazard or Injury

Use Proper Line Cord

Use only the specified line cord for this product and make sure line cord is certified for country of use.The operating voltage range for the BTX Gemini Twin Wave Series is 100-240 vac, 50-60 Hz.

Ground the Product

This product is grounded through the grounding conductor of the power cord. To avoid electric shock, the grounding conductor must be connected to earth ground. Before making any connections to the input or output terminals of the product, ensure that the product is properly grounded.

Make Proper Connections

Make sure all connections are made properly and securely. Any signal wire connections to the unit must be no longer than 3 meters.

Observe All Terminal Ratings

Review the operating manual to learn the ratings on all connections.

Use Proper Fuse

Use only specified fuses with product.

Avoid Exposed Circuitry

Do not touch any electronic circuitry inside of the product.

Do Not Operate with Suspected Failures

If damage is suspected on or to the product do not operate the product. Contact qualified service personnel to perform inspection.

Orient the Equipment Properly

Do not orient the equipment so that it is difficult to operate the disconnection device.

Place Product in Proper Environment

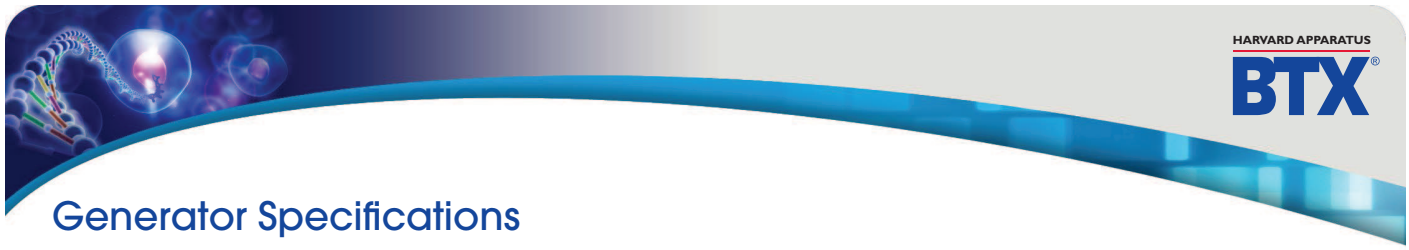
Review the operating manual for guidelines for proper operating environments.

Do Not Operate in Low Impedance Sample or Load

If the electroporation samples have an impedance of less than 20 Ω in LV and 40 Ω in HV, this may result in sample loss and potential damage to the unit.

Observe All Warning Labels on Product

Read all labels on product to ensure proper usage.



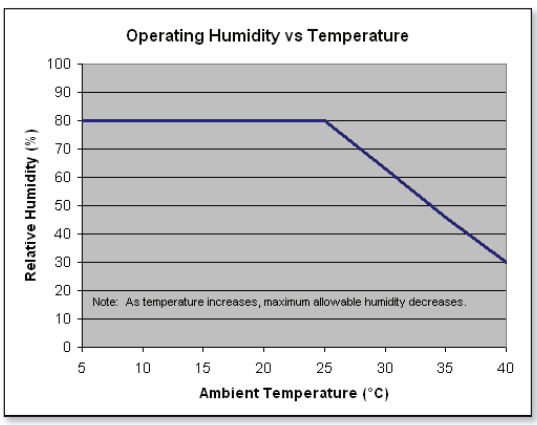
Generator Specifications

Generator Specifications (V7)	BTX Gemini SC²	BTX Gemini X²
Square Wave Pulse Ranges		
Voltage Range		
LV Mode	10 to 500 / 5 V res	5 to 500 / 1 V res
HV Mode	510 to 3000 / 10 V res	505 to 3000 / 5 V res
Voltage Accuracy		
LV Mode	5%	5%
HV Mode	5%	5%
Pulse Length Range		
LV Mode	50 µs to 10 ms / 50 µs res, 10 ms to 100 ms / 1 ms res	10 µs to 999 µs / 1 µs res, 1 ms to 999 ms / 1 ms res
HV Mode	50 µs to 5 ms / 50 µs res	10 µs to 600 µs / 1 µs
Multiple Pulsing		
LV Mode	1 to 10	1 to 120 (10 per sample)*
HV Mode	1 to 2	1 to 36 (3 per sample)*
Pulse Interval	LV : 0.1S to 10S	0.1 s to 10 sec
Capacitance in µF		
LV Mode	3775 µF	3775 µF
HV Mode	85 µF	85 µF
Voltage Droop	20% Load < 1 K Ω, 10% Load > 1 K Ω	20% Load < 1 K Ω, 10% Load > 1 K Ω
Exponential Decay Pulse Ranges		
Voltage Range		
LV Mode	10 to 500 / 5 V res	5 to 500 / 1 V res
HV Mode	510 to 3000 / 10 V res	505 to 3000 / 5 V res
Voltage Accuracy		
LV Mode	5%	5%
HV Mode	5%	5%
Time Constant Range		
LV Mode	1.25 ms to 3.275 S / 1.25 ms	1 ms to 5.158 S
HV Mode	0.5 ms to 50 ms / 0.5 ms	0.5 ms to 133.875 ms
Multiple Pulsing		
Multiple Pulsing	No	1 to 12 (Rint <100 ohm)*, 1 to 24 (Rint >100 ohm)*
Pulse Interval	N/A	5 to 30 sec
Capacitance in µF		
LV Mode	25 to 3275 µF / 25 µF res	25 to 3275 µF / 25 µF res
HV Mode	10, 25, 50 µF	10, 25, 35, 50, 60, 75, 85 µF
Internal Resistance (in parallel with load)		
All Modes	50 to 1000 Ω / 50 Ω res	LV 25 to 1575 Ω / 25 Ω res, HV 50 to1575 Ω / 25 Ω res
Sample Resistance (Load) Exponential Decay or Square		
LV Mode	Load ≥ 10 Ω	PL ≤ 100ms, load ≥ 8-9 Ω, PL >100ms, load ≥100 Ω
HV Mode	510-2500 Load ≥ 20 Ω, 2500 to 3000 V, Load ≥ 600 Ω	Load ≥ 40 Ω
Other Spcifications		
Charging Time	LV < 7 sec, HV < 4 sec	LV < 7 sec, HV < 4 sec
Monitoring	Pulse Voltage, Width, Droop % and Sample Resistance	Pulse Voltage, Width, Droop % and Sample Resistance
Display	4.3 in color display	4.3 in color display
Controls	Touch Screen	Touch Screen
Programmability	Storage over 1000 Protocols	Storage over 1000 Protocols
Arc Control	Yes	Yes

** When used in Plate Handler mode*

Generator Specifications

Generator Specifications (V7)	BTX Gemini SC²	BTX Gemini X²
Other Spcifications (continued)		
Interlock Switches	Yes	Yes
Pre-Pulse Sample resistance check	Yes	Yes
Pulse Over Current Protection	Yes	Yes
PC control	No	Yes
PC communications	No	Yes
Log Report	No	Yes
Remote Operation	No	Yes
Foot Switch Control	No	Yes
Power Ratings	35 W idle and 350 W pulsing	35W idle and 350 W pulsing
Input Voltage ratings	100 to 240 VAC	100 to 240 VAC
CE, ETL Marking, CB Scheme, EuRoHS	Yes	Yes
Dimensions (H x W x D) inches	12.75 x 11.25 x 8.5	12.75 x 11.25 x 8.5
Weight	~16 lb	~16 lb
Warranty	1 year	2 years
Atmospheric Specifications		
Operating Temperature	4°C to 40°C (40°F to 104°F)	
Storage Temperature	-10°C to 70°C (14°F to 158°F)	
Operating Humidity	See Chart Below	
Storage Humidity	20% to 80% RH, non condensing	
Mode of Operation	Continuous	
Classification	Class I	
Pollution	Degree 1	
Installation	Category II	
Supplier Name	Harvard Apparatus BTX	
Supplier Address	84 October Hill Rd., Holliston, MA 01746	
Supplier Phone Number	508-893-8999	
Regulatory Certifications	CE, ETL (UL, CSA), WEEE, EU RoHS & CB Scheme	
Safety Declarations	ANSI/UL 61010-1:2012 Ed. 3 Rev. 2012 ; CAN/CSA C22.2 No. 61010-1:2012 Ed. 3 ; IEC 61010-1:2001 Ed. 2 Corrigendum 1:2002, Corrigendum 2:2003 ; CENELEC EN 61010-1:2001 ; CB Scheme	
EMC Declarations	IEC 61326-1 Ed. 1	
FCC	47 CFR Part 15	



A Message from Harvard Apparatus BTX

Thank you for investing in a Harvard Apparatus BTX Gemini System.

Since its founding in 1983, BTX's main focus has been in the area of applying controlled electic fields for genetic engineering applications. Because of this, we quickly established a reputation as the technological leader in the fields of electroporation and electrofusion. Our systems have been installed in many prestigious institutes around the globe where they are used successfully for high efficiency transfection, transformation and cell fusion applications. We offer a variety of waves forms, electrodes and chamber options to provide, you, the researchers, with the tools to achieve your goals.

We are vested in your success. To that end, the Harvard Apparatus BTX technical support team constantly tracks published literature for any reference to electroporation and electrofusion. We extract the pertinent experimental conditions and yields from these papers to help us in our efforts to help you. In addition to tracking publications, we are available to you for support at any time for advice in experimental design, product recommendations, troubleshooting, and any other relevant technical advice.

We thank you again for your investment and we look forward to assisting you in any way we can.

Finally, please read this manual carefully before attempting to operate the electroporation system. If you have any questions about the unit or about particular applications, please contact us:

Harvard Apparatus BTX
84 October Hill Rd
Holliston, MA 01746 USA

Toll Free: 1-800-272-2775
International Callers: 508-893-8999
Fax: 508-429-5732
Web: www.btxonline.com
Email: techsupport.btx@harvardapparatus.com

For any customers outside the US or Canada, please call your local Harvard Apparatus BTX dealer or call us directly.

Electroporation Overview

DNA Delivery Into Cells Using Electroporation

General Electroporation Discussion

Electroporation is the use of a transmembrane electric field pulse to induce microscopic pathways (pores) in a bio-membrane. Their presence allows molecules, ions, and water to pass from one side of the membrane to the other. When the electric field is applied the ions inside and outside the cell membrane migrate. As the charge builds up on either side of the membrane the membrane weakens and the pathways form permitting material outside of the cell to enter. If the electric field is promptly removed the pathways close and the membrane reseals. If the electric field duration is too long the pathways increase and the cell is killed. Efficient electroporation depends on proper selection of electric field waveforms. The electropores are located primarily on the membrane areas which are closest to the electrodes. The pathways form in about a microsecond and seal in seconds to minutes. The duration of the electric field is tens of microseconds to tens of milliseconds.

The use of electroporation was described by Neumann in the early 1980's. The routine use of electroporation became very popular with researchers through the 1980's because it was found to be a practical way to place drugs, or other molecules into cells. In the late 1980's, scientists began to use electroporation for applications in multi-cellular tissue.

In the early 1990's Lluis Mir of the Institute Gustave-Roussy was the first to use electroporation in a human trial to treat external tumors.

Research has shown that the induction of pathways is affected by three major factors. First, cell-to-cell biological variability causes some cells to be more sensitive to electroporation than other cells. Second, for pathways to be induced, the product of the pulse amplitude and the pulse duration has to be above a lower limit threshold. Third, the number of pathways and effective pathway diameter increases with the product of "amplitude" and "duration." Although other factors are involved, this threshold is now understood to be largely dependent on a fourth factor, the reciprocal of cell size. If the upper limit threshold is reached pore diameter and total pore area are too large for the cell to repair by any spontaneous or biological process, the result is irreversible damage to the cell or cell lysis. Because the mechanism of electroporation is not well understood, the development of protocols for a particular application has usually been achieved empirically, by adjusting pulse parameters (amplitude, duration, number, and inter-pulse interval).

Research shows that certain experimental conditions and parameters of electrical pulses may be capable of causing many more molecules to move per unit time than simple diffusion. There is also good evidence (Sukharev et al., 1992) that DNA movement is in the opposite direction.

An additional important consideration is when the voltage pulse is applied to the cells and medium that the amount of current that flows is dependent on the conductivity of the material in which the cells are located. Some material is quite conductive and severe heating will occur if the pulse duration is too long. Therefore long duration fields will kill cells by destroying the membrane and heating.

The electric field in which the cells are located is produced by two system components. The first is the voltage waveform generator and the second is the electrode which converts the voltage into the electric field.

As the charge accumulates at the membrane, which is a capacitance, the voltage across the membrane increases.

voltage = capacitance charge

As charge accumulates at the membrane, the voltage across the membrane increases. Neumann et al. (1989) described the equation that relates the transmembrane voltage (TMV) to electric field intensity:

$$TMV\ 3/2 = E\ r\ |\ \cos \alpha|$$

where:

E = electric field intensity in volts/cm
r = the cell radius in cm
α= angle off the center line

Pores in the membrane will begin to form as the voltage increases from its quiescent value of a few tenths of a volt to more than 0.5 volts. To produce a TMV of 1 volt across the membrane of a cell with 7μm radius, the required electric field intensity is:

$$E = \frac{2}{3} * \frac{1}{7 \times 10\text{-}4} = 950 \text{ volts/cm}$$

The number of pores and effective pore diameter increase as the product of pulse amplitude and duration increase. At the upper limit threshold, pore diameter and total pore area become too large for the cell to repair by any spontaneus or biological process. The result is irreversible damage to the cell or cell lysis.

Another important point to consider is the generation of heat during electroporation. Heat production is directly related to current intensity which is, in turn, dependent on the conductivity of the material through which the electric field is applied. Standard saline solutions such as PBS and many tissue culture media are highly conductive and, thus. will generate considerable amounts of heat when used in cell electroporation. Excessive heating can be detrimental to cell viability. The effects of heating can be reduced by using a low conductivity medium such as BTX's Cytoporation medium to resuspend cells prior to electroporation.

Although electroporation is an effective method for introducing macromolecules onto cells, the biological mechanisms by which cells become electroporated are not completely understood. Therefore, the development of specific protocols for particular applicatons is usually achieved by empirical adjustment of pulse parameters (i.e. amplitude, duration, pulse number, and interpulse interval).

Glossary of Terms

Capacitance – The ability of a body to store an electrical charge. Any object that can be electrically charged exhibits capacitance.

Capacitor – A device that stores energy in the form of an electric field. A capacitor consists of two metal plates insulated from each other by a dielectric (insulating, usually a plastic material such as Mylar) material. In an ideal capacitor, no conduction current flows between the plates after the capacitor is completely charged. Capacitors can be fixed, variable, or adjustable.

Cell Form – The format in which cells present for experimentation. Cell forms include suspension, in vivo, in ovo, ex plant, adherent, whole organism, etc.

Dielectric Breakdown – The reversible breakdown of bi-lipid layer membranes as a result of the application of a DC electroporation pulse. A sufficiently high field strength may increase the membrane potential past a critical point leading to the breakdown of the membrane.

Dielectric Constant – For a given dielectric (nonmetallic) material, the ratio of electrical capacitance of a dielectric-filled capacitor to a vacuum capacitor of identical dimensions.

Divergence – The deviation of field lines (e.g. electric field lines) from parallel, homogeneous conditions. A highly divergent field is a very inhomogeneous field where the value and direction of the field change drastically in the area under consideration.

Electrolytic – A fluid containing charged molecules is called an electrolyte. Electrolytic properties are associated with such a fluid, such as the ability to conduct current.

Electroporation – The application of high electric field pulses of short duration to create temporary pores (holes) in the membranes of cells.

Electroporation Cuvette – Square chambers with electrodes on two sides, usually measuring 1 mm, 2 mm or 4 mm in gap, for the purpose of electroporating cells in suspension. The cell type being electroporated typically determine the gap. Prokaryotic cells typically uses 1 mm or 2 mm gap cuvettes, while eukaryotic cells will typically use 2 mm or 4 mm gap sizes.

Exponential Decay Waveform – This waveform is mainly used for transforming cells during electroporation. In this type of pulse the set voltage is released from the capacitor and decays rapidly and exponentially over time (millisecs). The delivered pulse, is characterized by two parameters: the field strength (kV/cm) and the time constant. These parameters can be adjusted by varying voltage and capacitance settings to achieve a wide pulse gradient.

Field Strength – The potential difference between two points (electrodes) (in Volts) divided by the distance between the electrodes (called gap, and expressed in cm). Expressed as V/cm or kV/cm. This is true only if the electric field is homogenous as it is in parallel plate electrodes.

Gap – The distance between electrodes.

Homogenous Electric Field – The direction and field strength are constant.

Hydrostatic Pressure – The pressure in liquids at rest.

Inhomogeneous Electric Field – Direction and strength of the electric field vary.

Number of Pulses – The number of pulses the sample will be exposed to.

Osmotic Pressure – The applied pressure required to prevent the flow of solvents of different concentration across a semi-permeable membrane.

Pore – A small, mostly transient opening in a cell wall caused by the application of a brief high electric field pulse.

Potential Difference – The difference (in Volts) between points in an area between electrodes.

Protocols – The method for performing an experiment.

Pressure Gradient – The difference in pressure between two points in a medium.

Pulse Interval – The time between multiple pulses.

Pulse Length/Pulse Duration – The length of time an electric signal is applied.

Specialty Electrodes – Electrodes used with the BTX Gemini X^2 to perform electroporation on a wide variety of cell forms. Some applications include in vivo, in ovo, in utero, or ex plant electroporation. Harvard Apparatus BTX offer many types of specialty electrodes.

Square Waveform – This waveform is typically used for eukaryotic cells. It is is characterized by the voltage delivered, the duration of the pulse, the number of pulses and the length of the interval between pulses.

Time Constant – (represented by the greek letter tau, T) is the amount of time required for the actual voltage of the delivered pulse to decrease to a value 1/e of the true peak pulse.

Transfection – The introduction of nucleic acids into animal cells. Stable transfections result in integration of nucleic acids into host chromosomes and the inheritance of associated traits in progeny cells. Transient transfections result in temporary expression of exogenous nucleic acids.

Transformation – The introduction of nucleic acids into microorganisms and plant cells.

Turgor Pressure – The pressure in capillaries.

Voltage – The difference of electric potential between two electrodes (expressed in volts (V) or kilovolts (kV)).

Waveforms – The shape of time-varying electric signals.

Product Overview

The **BTX Gemini X²** is a highly advanced twin waveform generator incorporating both square and exponential decay waves in a single unit. The BTX Gemini X² has been designed with these waveform combinations to enable researchers to easily and efficiently electroporate eukaryotic cells and prokaryotic cells in all forms with one easy to use setup. The BTX Gemini X², which can be operated via PC or remote control, boasts a wide range of voltage (5-3000 v, 1 v or 5 v increments), pulse length (10 μS – 1S, 1 μS resolution), time constant options (which include capacitance choices from 25 to 3275 μF in LV and 10, 25, 35, 50, 60, 75, 85 μF in HV), along with multiple pulsing options with both the square waveform and the exponential decay waveform, and unparalleled pulse delivery accuracy. The BTX Gemini X², with over 1,000 custom protocol storage, monitors and displays pre-pulse sample resistance as well as delivered voltage values and records logs of all experiment parameters internally, which can be downloaded to a computer for analysis and QC. The generator is controlled through a color LCD touch screen interface and incorporates USB communications. The pulse can also be activated by a foot switch. The enhanced safety features of the BTX Gemini X² protect users as well as precious samples. The BTX Gemini X² is designed to give researchers the ultimate flexibility in their experiments, making it possible to perform electroporation on tissues and organs in vivo (as well as in utero, in ovo, ex plant) on adherent cells, and cells in suspension in either single cuvettes or 96 well plates. The Safety Dome allows researchers to safely work with electroporation cuvettes, up to two at a time, giving researchers the ability to experiment on sample volumes from 20 μl up to 800 μl. This state of the art system comes complete with the twin waveform generator for cell electroporation in all forms, dozens of preprogramed protocols for commonly electroporated cells, Safety Dome, 30 cuvettes, cuvette stand, user manual, two year warranty, unlimited application support and the same high quality researchers have come to expect from Harvard Apparatus BTX.

The **BTX Gemini SC²** is a twin waveform generator incorporating both square and exponential decay waves in a single unit. These waveform combinations enable researchers to easily and efficiently electroporate eukaryotic and prokaryotic cells in suspension with one easy to use setup. The BTX Gemini SC² boasts a wide range of voltage (10-3000 v, 5 v or 10 v increments), pulse length (50 μS – 5 mS, 50 μS resolution), time constant options (which include capacitance choices from 25 to 3275 μF in LV and 10, 25 or 50 μF in HV), multiple pulsing option with the square waveform and unparalleled pulse delivery accuracy. The BTX Gemini SC², with unlimited custom protocol storage, monitors and displays pre-pulse sample resistance as well as delivered voltage values. The generator is controlled through a color LCD touch screen interface. The enhanced safety features of the BTX Gemini SC² protect users as well as precious samples. The Safety Dome allows researchers to safely work with electroporation cuvettes, up to two at a time, giving researchers the ability to experiment on sample volumes from 20 μl up to 800 μl. This affordable system comes complete with the dual waveform generator for suspension cell electroporation, dozens of preprogramed protocols for commonly electroporated cells, Safety Dome, 30 cuvettes, cuvette stand, user manual, two year warranty, unlimited application support and the same high quality researchers have come to expect from Harvard Apparatus BTX.

What is the difference between the Gemini X² and the Gemini SC²?

The Gemini X² is designed to give researchers the ultimate control and flexibility in their experiments by making it possible to perform electroporation on tissues and organs in vivo (as well as in utero, in ovo, ex plant) on adherent cells, as well as cells in suspension in either single cuvettes or 96 well plates, can be controlled remotely via footswitch or PC, and it offers storage of pulse data. Because of the Gemini X² broad range of use, the specifications are wide-ranging, making the Gemini X² the most versatile electroporation system available today.

The Gemini SC² is designed for researchers working to electroporate cells in suspension in cuvettes. This system cannot accommodate multiple pulsing with the exponential decay waveform, remote operation, specialty electrodes or 96 well options. For this reason, some of the specifications of the Gemini SC² are not as extensive as what is available in the Gemini X².

Unpacking the System

The shipping carton in which your BTX Gemini Twin Waveform Electroporation System is packed has been specifically designed to provide maximum protection to the instrument during transportation and normal handling conditions. Upon receipt, the carton should be examined for any external damage resulting from shipment.

Open the carton and carefully remove the BTX Gemini Twin Waveform Electroporator and inspect the unit for any apparent damage. Save the carton and packing materials for future transportation and shipping requirements.

Packing Data

Check the packing slip to ensure that all items ordered and listed are included in the shipment. Inform Harvard Apparatus BTX immediately if any parts are missing or damaged.

Power Source

As received, the instrument is ready for use with either 100-240 V AC, 50/60 HZ.

The power requirements are 350 watts. In the USA, the power cord has a standard three prong plug.

Installation

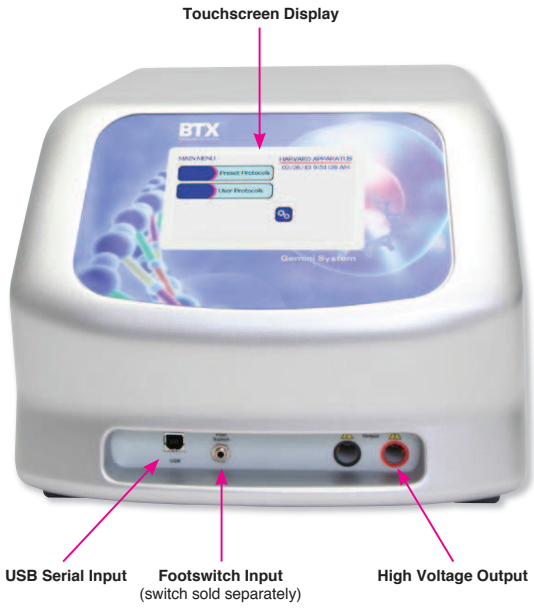
Once you have determined that the components of the system have not sustained any obvious damage in shipment, proceed with the installation. The location of the BTX Gemini Twin Waveform Electroporator should be a dry, level, sturdy surface free from extremes in ambient temperature, dust or chemical exposures. Unpack the safety dome, cuvette rack and disposable cuvette chambers.

Connect the safety dome or in the case of the BTX Gemini X^2 system, specialty electrodes or HT plate handler, into the connectors at the bottom right-hand side of front panel.

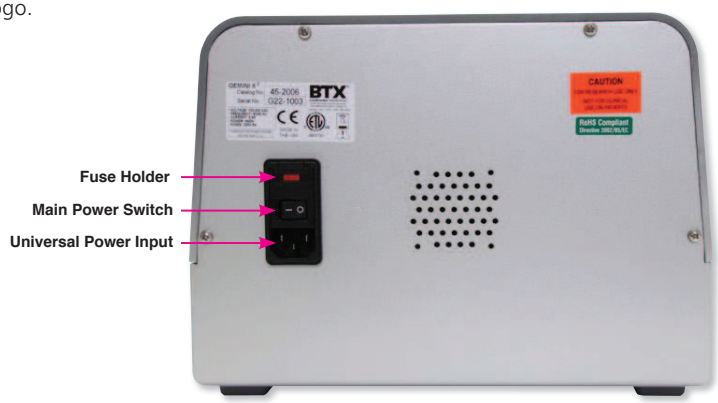
Connect the mains/power cord to into the back panel at the bottom left.

Power up the system by pushing the rocker switch located on the back panel at the bottom left. The display will flash the BTX logo. Once the software initializes, the Home screen will appear.

You are now ready to begin your work.



Gemini SC²

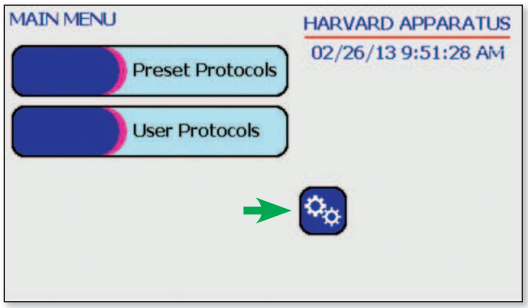


Operating the System

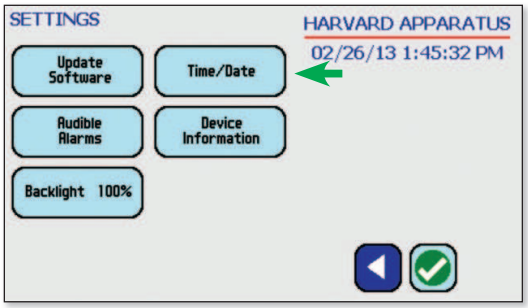
Setting Time/Date

After initializing, the home screen will appear.

Tap the Gears icon



Tap Time/Date icon.

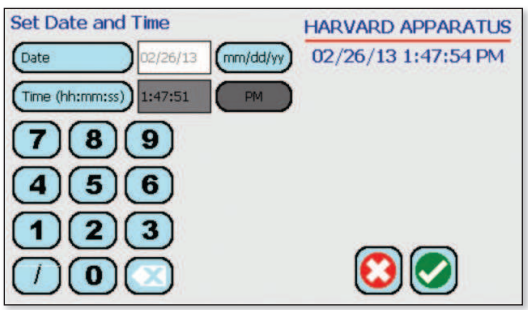


Tap Date icon and use the keypad to type the date.

Tap Time icon and use the keypad to type the time.

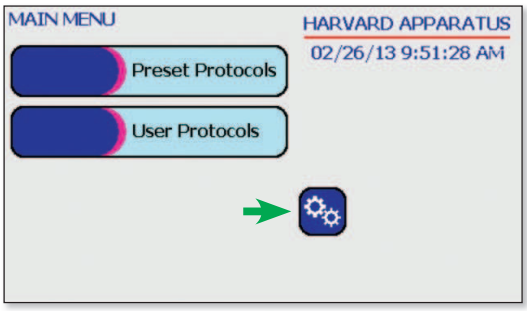
Tap the Green Check icon to save and return to the settings screen.

NOTE: You may change the date and time format by tapping icons to the right of the given values.

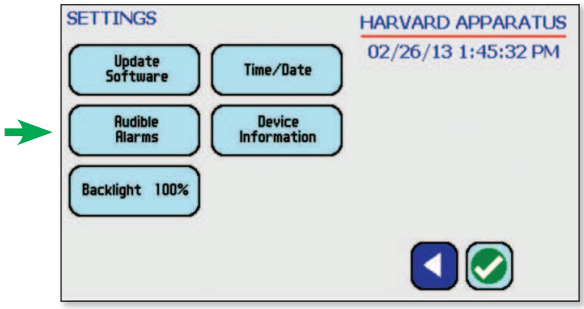


Setting Audible Alarm Preferences

On the home screen, tap the Gears icon

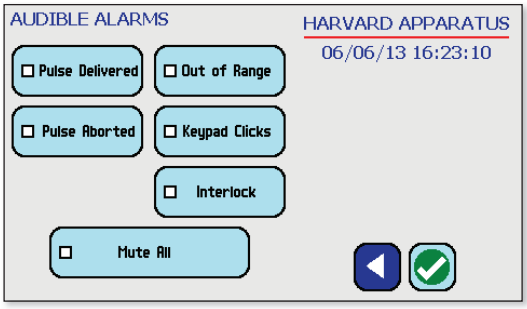


Tap Audible Alarms icon.



Select preferred audible alarms by tapping the icons to activate or deactivate.

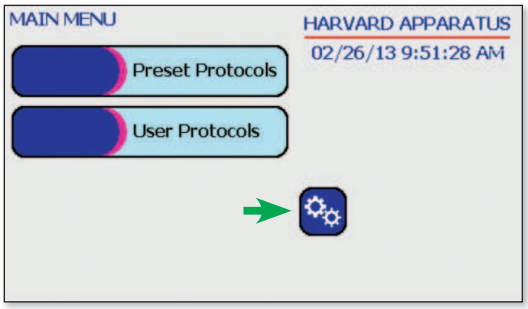
Tap the Green Check icon to save and return to the settings screen.



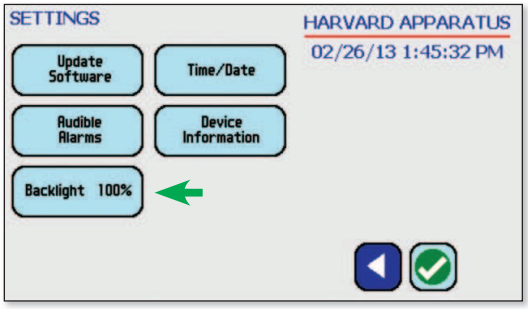
Operating the System

Setting Backlight Preferences

On the home screen, tap the Gears icon

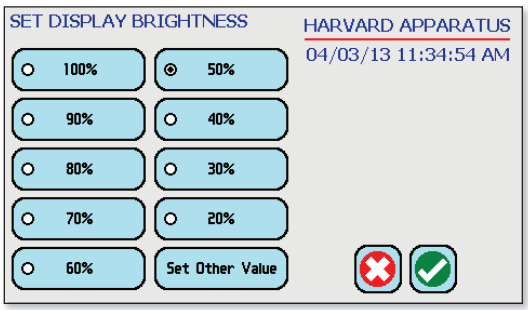


Tap Backlight icon.



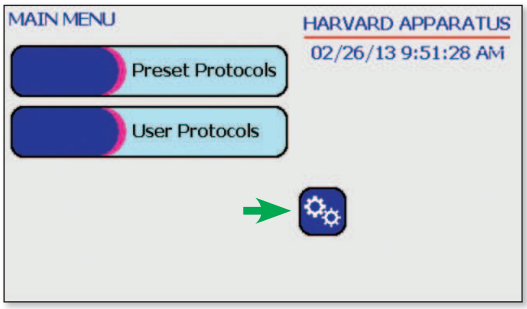
Select preferred brightness by tapping the icons indicating % brightness.

Tap the Green Check icon to save and return to the settings screen.

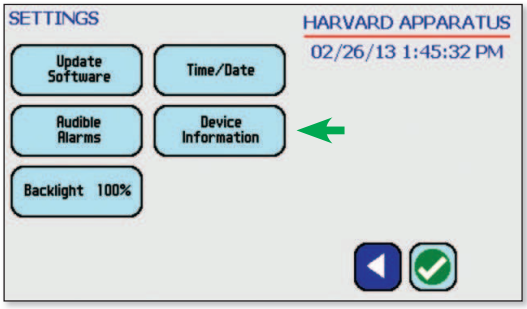


Displaying Device Information

On the home screen, tap the Gears icon.

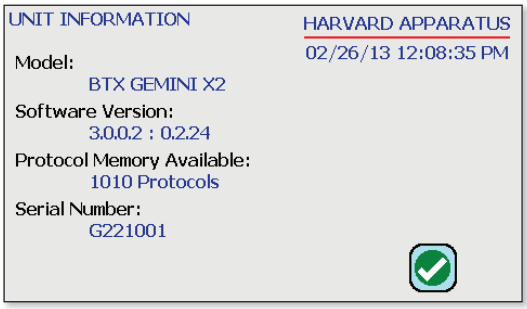


Tap Device Information icon.



View device information.

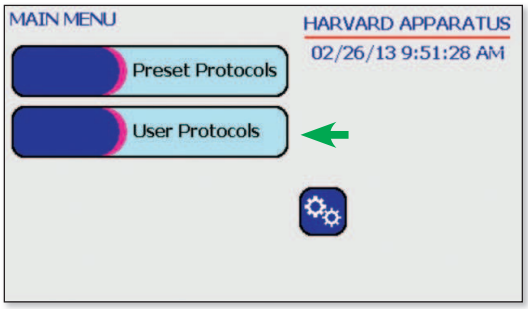
Tap the Green Check icon to return to the settings screen.



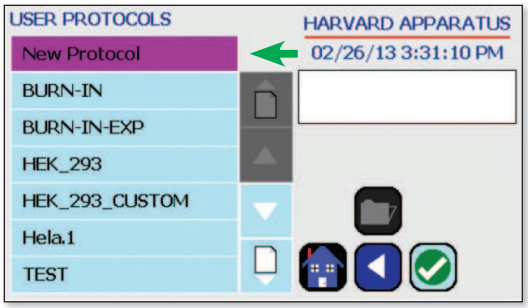
Performing Experiments

New Protocols

On the home screen, tap User Protocols icon.

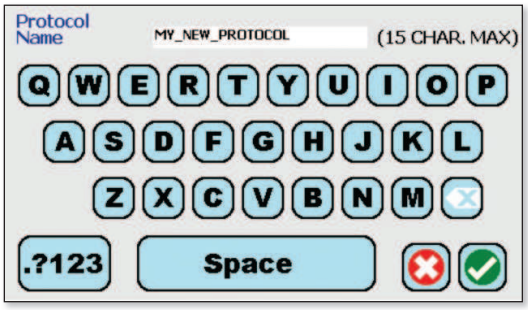


Double tap New Protocol.



Use keyboard to name protocol.

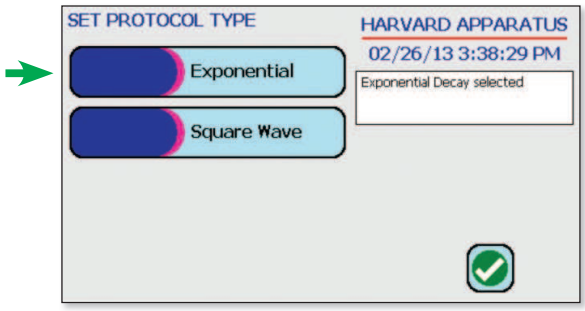
Tap the Green Check icon to save.



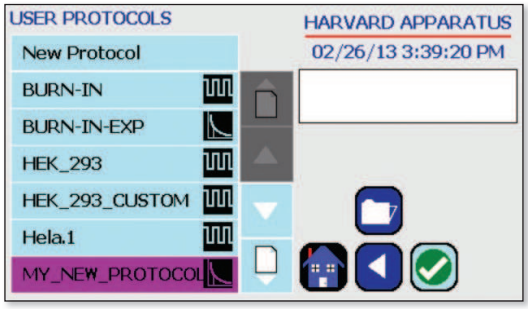
Exponential Decay Wave

Tap the Exponential icon.

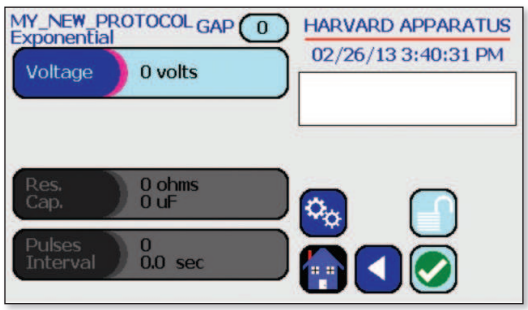
Tap the Green Check icon to save.



Double tap your newly saved protocol.

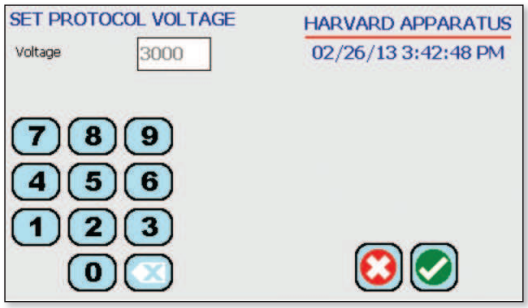


Tap the Voltage icon.



Use the numeric keypad to set voltage.

Tap the Green Check icon to save.

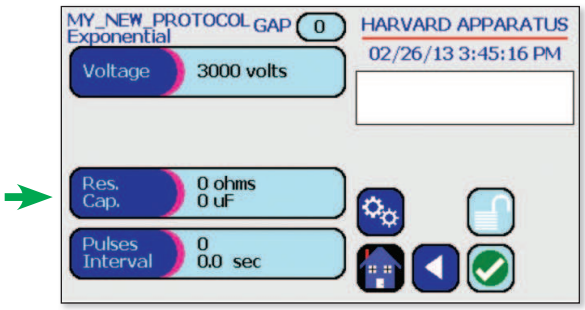


Performing Experiments

New Protocols (continued)

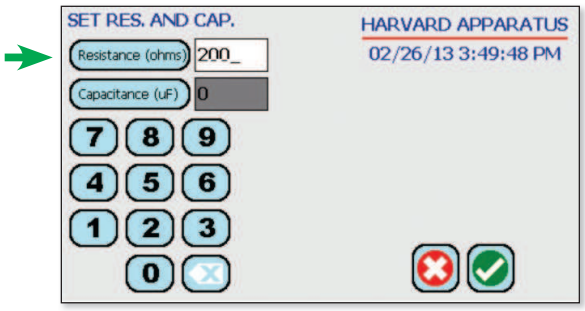
Exponential Decay Wave (continued)

Tap the Res. Cap. icon.



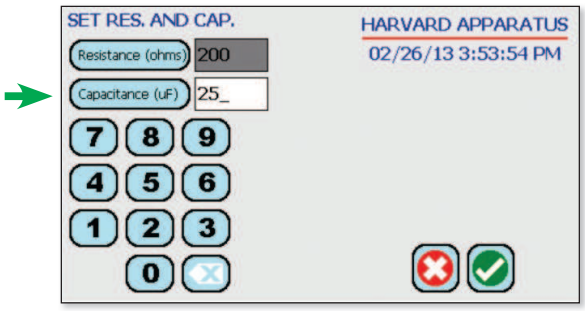
Tap the Resistance (ohms) icon.

Use the numeric keypad to set resistance value.

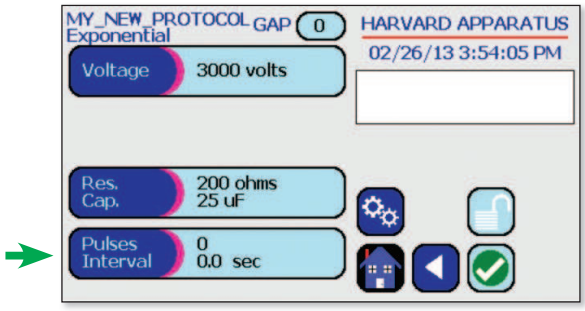


Tap the Capacitance (uF) icon.

NOTE: LV and HV mode capacitance values differ. Use the numeric keypad to set resistance value.



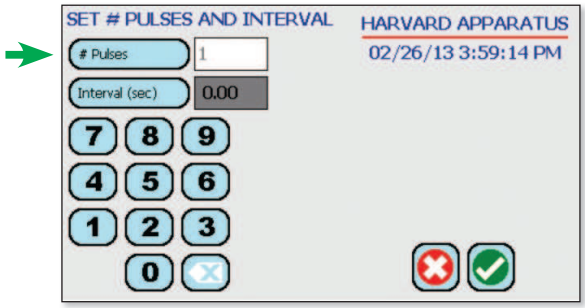
Tap the Pulses Interval icon.



Tap the # Pulses icon.

Use the numeric keypad to set the # of pulses.

NOTE: Only the BTX Gemini X² can perform multiple pulsing with Exponential Decay Wave, the BTX Gemini SC² cannot perform multiple pulsing with Exponential decay waves.

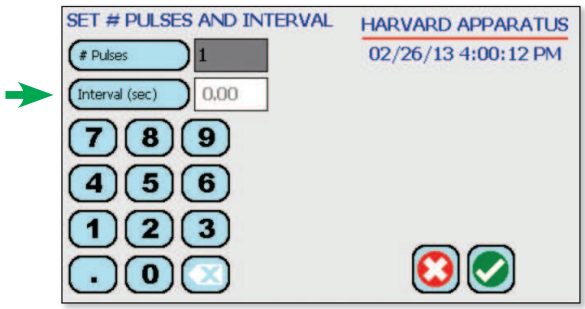


Tap the Interval (sec) icon.

Use the numeric keypad to set the interval between pulses.

Tap the Green Check icon to save.

NOTE: Intervals must only be set when performing multiple pulsing.

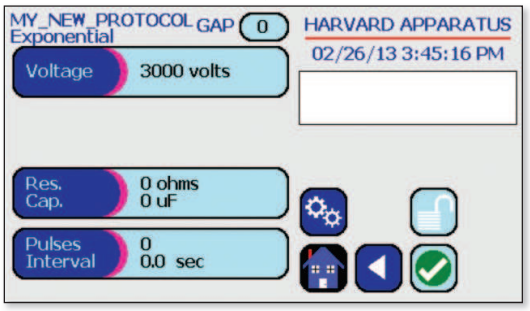


Performing Experiments

New Protocols (continued)

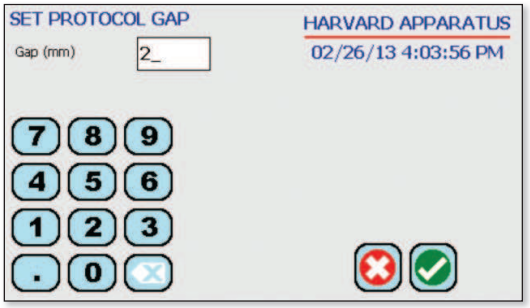
Exponential Decay Wave (continued)

Tap the Gap icon.

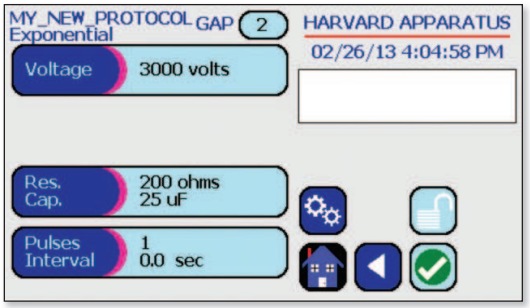


Use the numeric keypad to set the gap between electrodes.

Tap the Green Check icon to save.

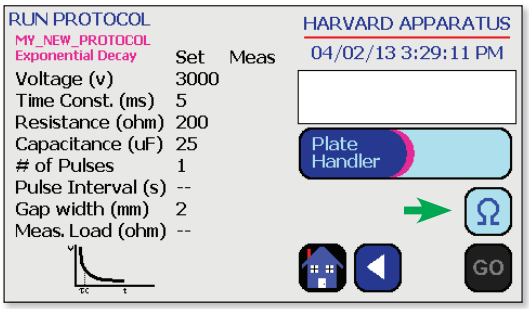


Tap the Green Check icon to proceed to the run screen.

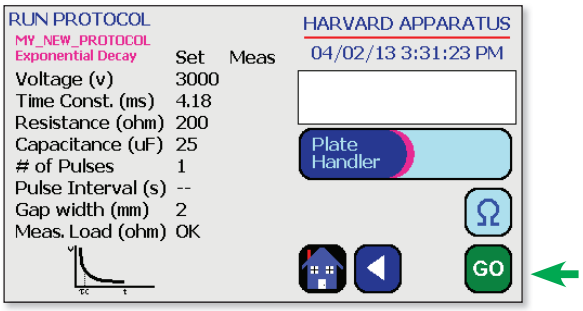


View your protocol.

To run, tap the Omega icon.



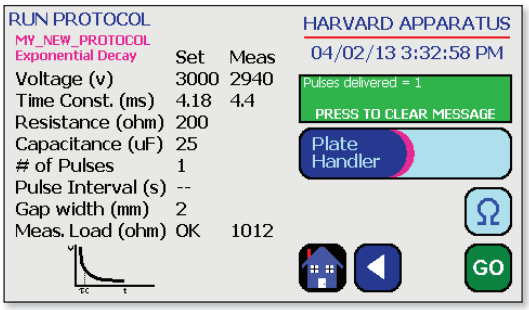
With load measurement OK, press the Go icon to run protocol.



Once protocol is complete, data regarding your pulse is displayed and stored in the systems logs (BTX Gemini X² only) for future use.

You may continue pulsing, go back to set-up screen, or to the home screen.

NOTE: See the Protocol Manager user manual for instructions on viewing and downloading log data.

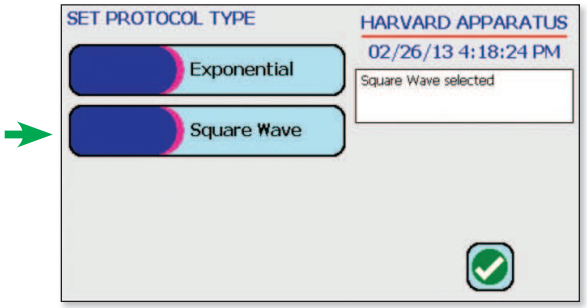


New Protocols (continued)

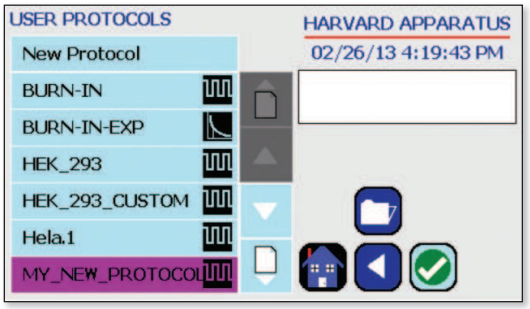
Square Wave

Tap the Square Wave icon.

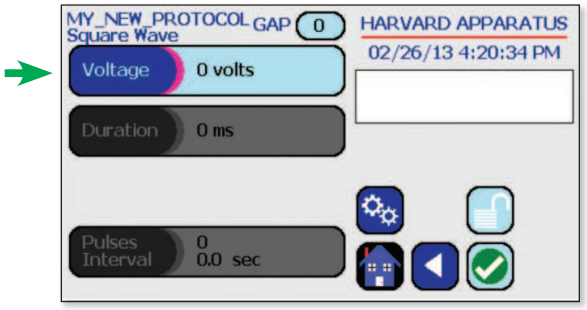
Tap the Green Check icon to save.



Double tap your newly saved protocol.

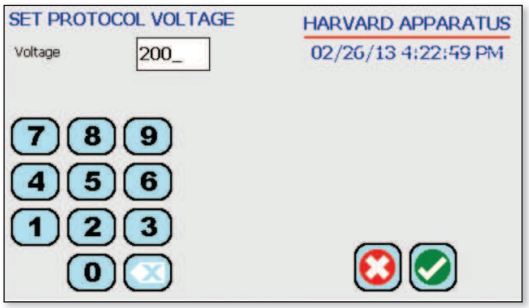


Tap the Voltage icon.

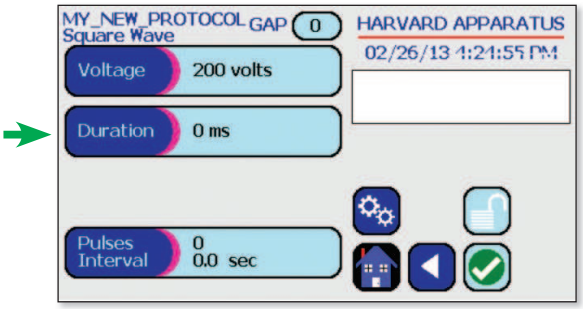


Use the numeric keypad to set voltage

Tap the Green Check icon to save.

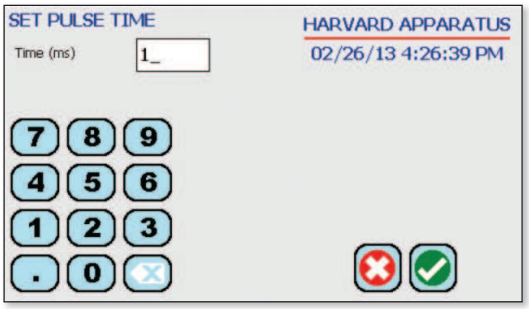


Tap the Duration icon.



Use the numeric keypad to set the pulse length.

Tap the Green Check icon to save.

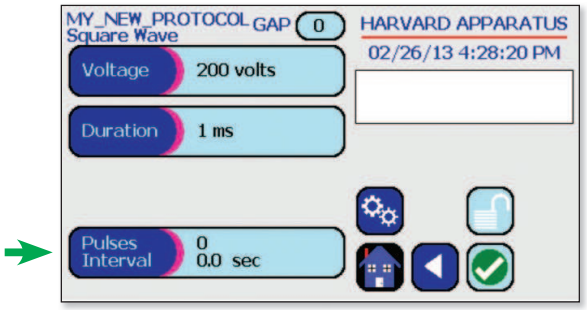


Performing Experiments

New Protocols (continued)

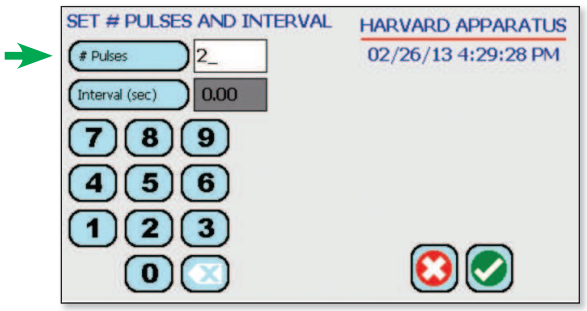
Square Wave (continued)

Tap the Pulses Interval icon.



Tap the # Pulses icon.

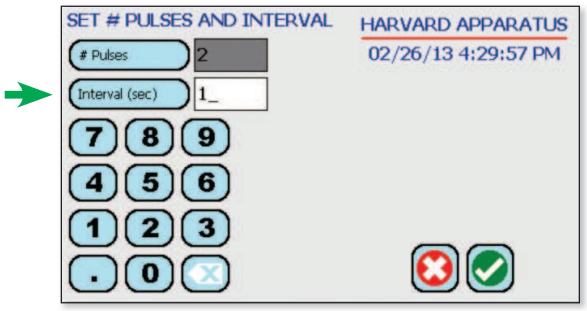
Use the numeric keypad to set the # of pulses.



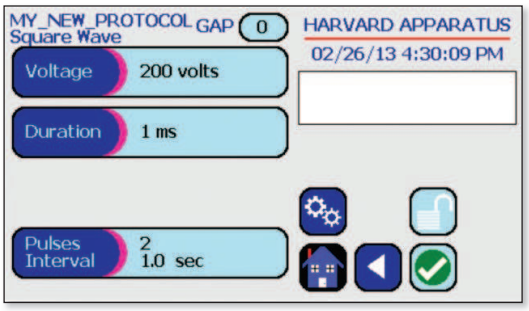
Tap the Interval (sec) icon.

Use the numeric keypad to set the pulse interval.

Tap the Green Check icon to save.

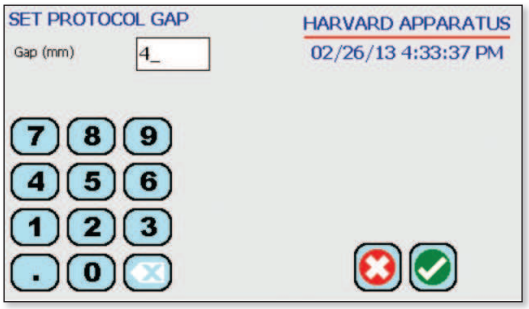


Tap the Gap icon

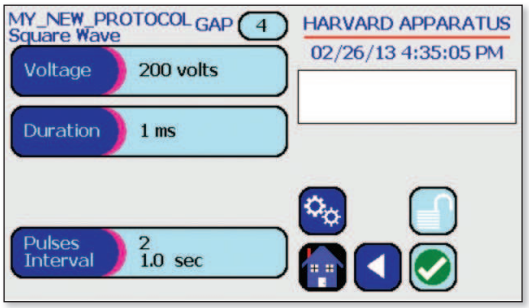


Use the numeric keypad to set the gap between electrodes.

Tap the Green Check icon to save.



Tap the Green Check icon to proceed to the run screen.



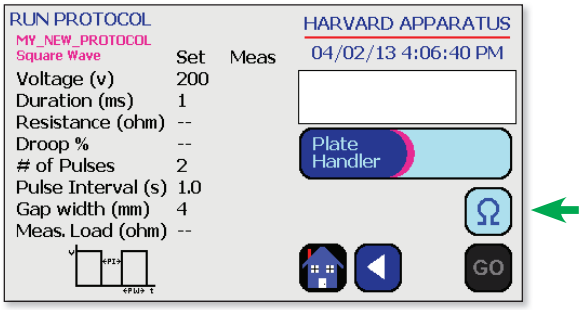
Performing Experiments

New Protocols (continued)

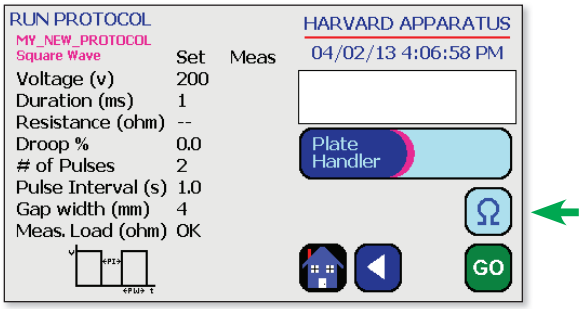
Square Wave (continued)

View your protocol.

To run, tap the Omega icon.

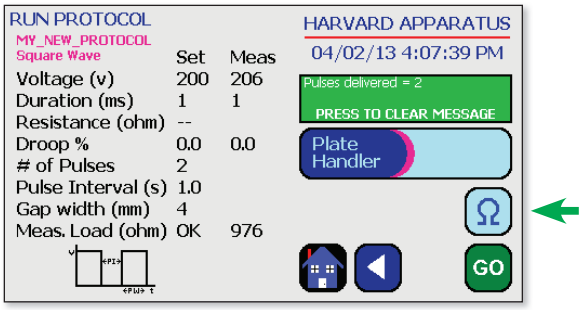


With load measurement OK, press the Go icon to run protocol.



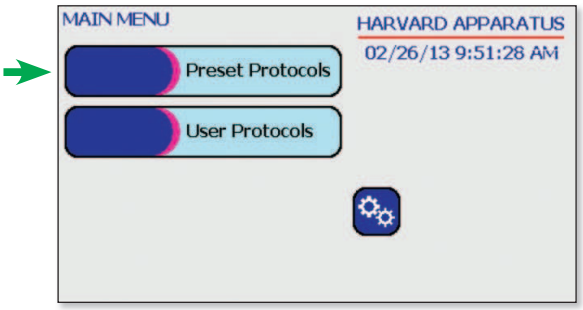
Once protocol is complete, data regarding your pulse is displayed and stored in the systems logs (BTX Gemini X^2 only) for future use.

You may continue pulsing, go back to the set-up screen, or to the home screen.

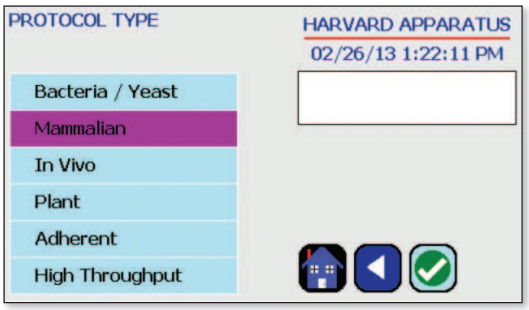


Preset Protocols

On the home screen, tap the Preset Protocols icon.

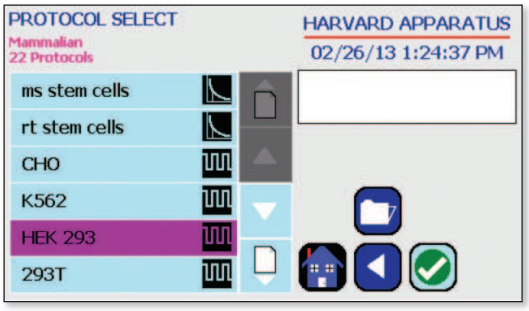


Double tap desired cell type.



Scroll through available cells.

Double tap desired cells.

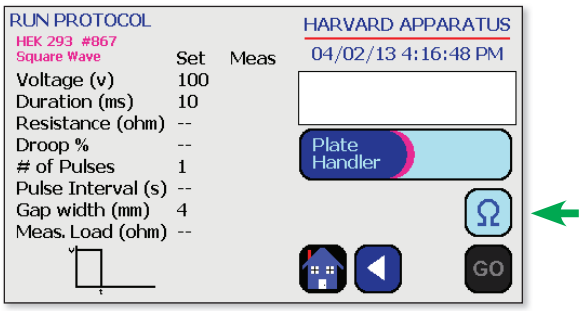


Performing Experiments

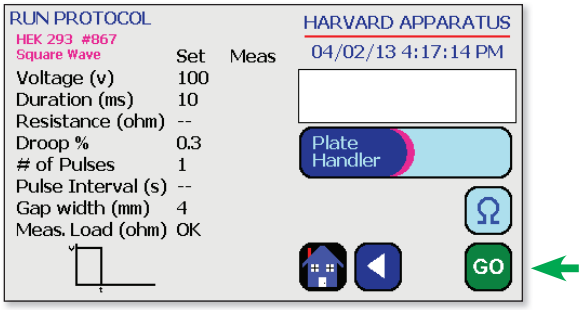
Preset Protocols (continued)

Review parameters.

Tap Omega icon to measure pre pulse load resistance.

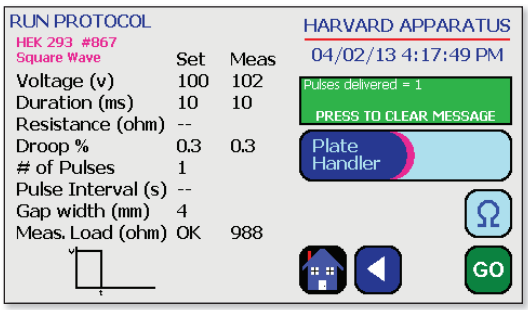


With load measurement OK press the Circle icon to run protocol.



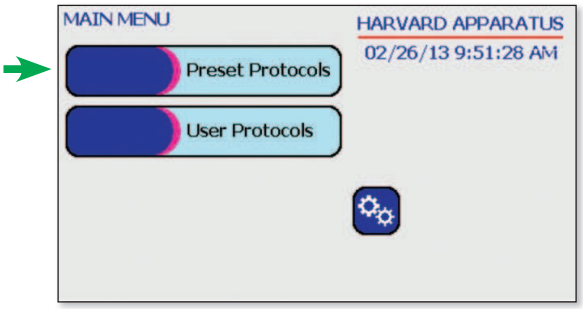
Once protocol is complete, data regarding your pulse is displayed and stored in the systems logs (BTX Gemini X² only) for future use.

You may continue pulsing, go back to the protocol select screen, or to the home screen.

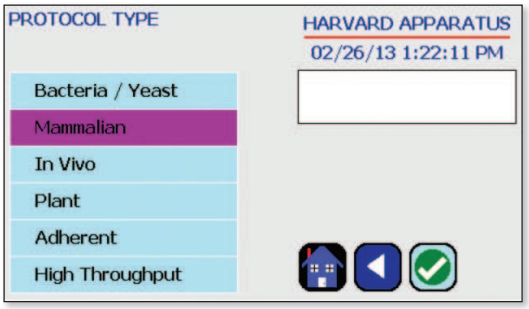


Customizing a Preset Protocol

On the home screen, tap the Preset Protocols icon.



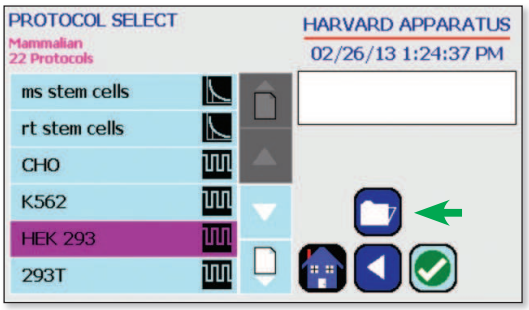
Double tap desired cell type.



Scroll through available cells.

Tap desired cells.

Tap Folder icon.

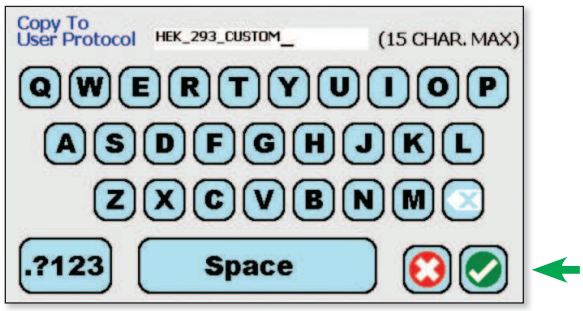


Performing Experiments

Customizing a Preset Protocol (continued)

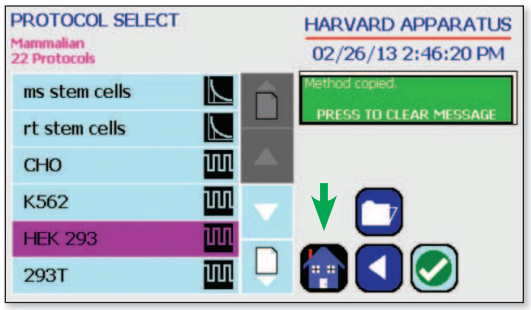
Use keyboard to name copy of protocol.

Tap the Green Check icon to save.

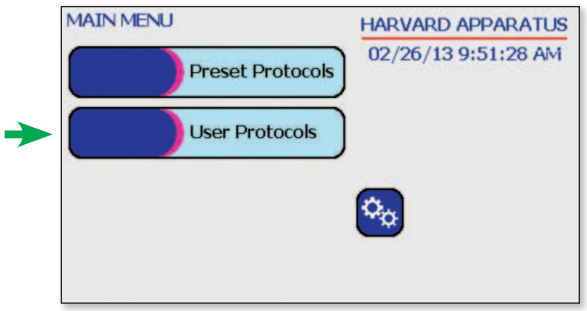


The message board will turn green and alert the user that the method has been copied.

Tap the Home icon.

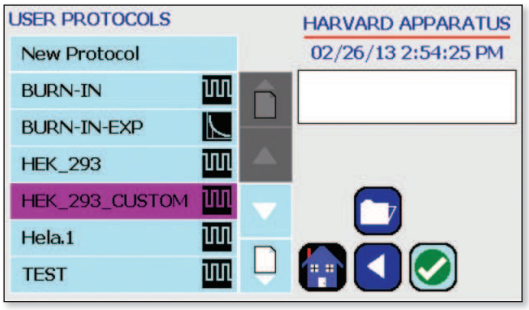


On the home screen, tap User Protocols icon.



Scroll through user protocols to locate the newly saved method.

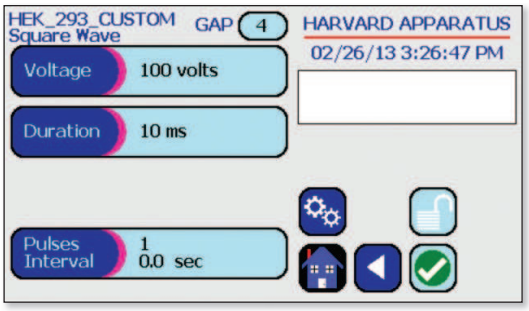
Double tap the newly saved method.



Tap the parameter(s) requiring customization.

Proceed to modify selected parameters.

Tap the Green Check icon to proceed to the run screen.

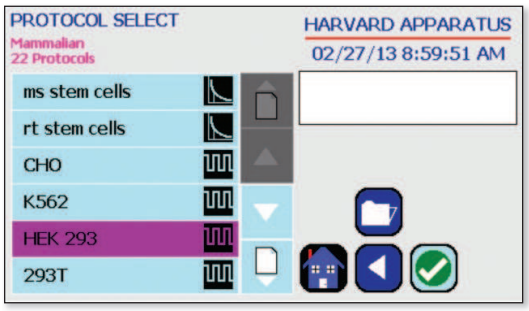


Using an HT Plate Handler (BTX Gemini X² Only)

Plug the HT Plate Handler into the front ports of the BTX Gemini X².

Scroll through available cells.

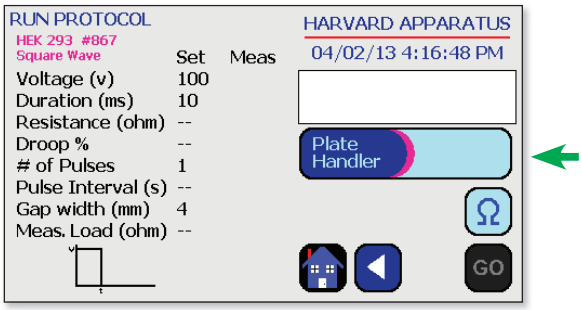
Double tap desired cells.



Using an HT Plate Handler (BTX Gemini X² Only) (continued)

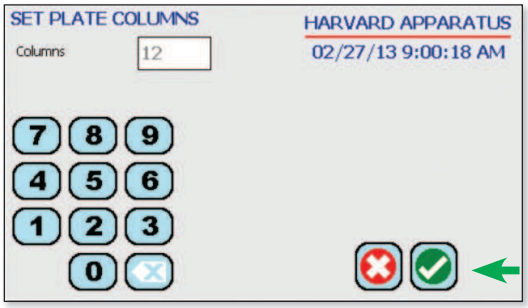
Review parameters.

Tap Plate Handler icon.

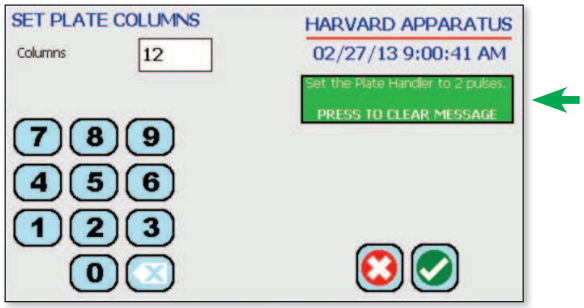


Using the numeric keypad icon, type the number of columns being pulsed.

Tap the Green Check icon to save.



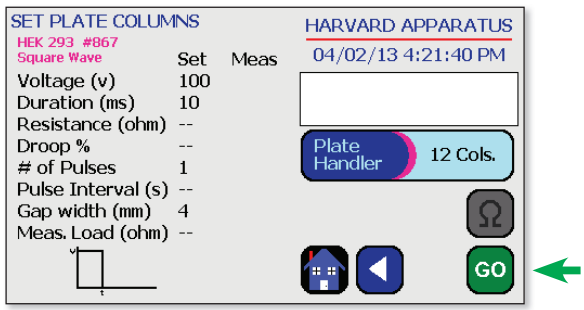
NOTE: Note the message box. It will indicate how many pulses should be set in the HT plate handler. This number will be a combination of both pre pulse resistance measurements and DC pulses.



Review parameters.

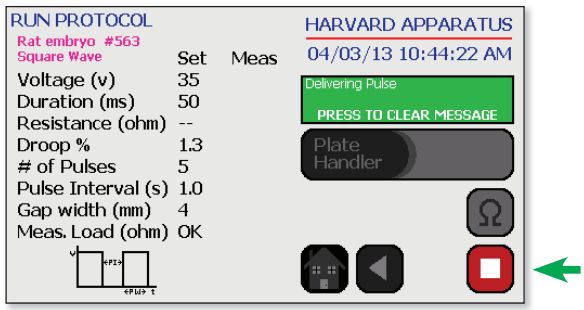
Set the HT plate handler with the correct number of pre-pulse resistance measurement and DC pulses.

Tap the Go icon to run protocol.



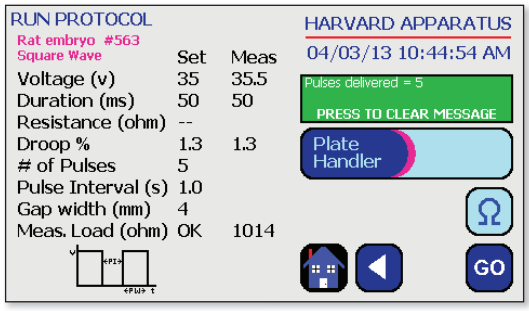
The Square icon can be used to stop the protocol at any time.

NOTE: Generator will display readings of the 1st and last pulse of each column.



Once protocol is complete, data regarding your pulse is displayed and stored in the systems logs for future use.

You may continue pulsing, go back to the settings screen or protocol select screen, or to the home screen.



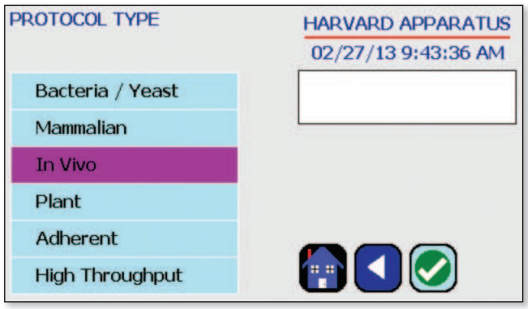
Performing Experiments

Using Specialty Electrodes (BTX Gemini X² only)

Plug the specialty electrodes into the front ports of the BTX Gemini X²

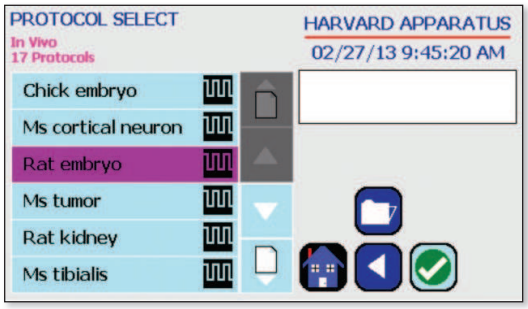
Scroll through available protocols.

Double tap desired selection.



Scroll through available cells.

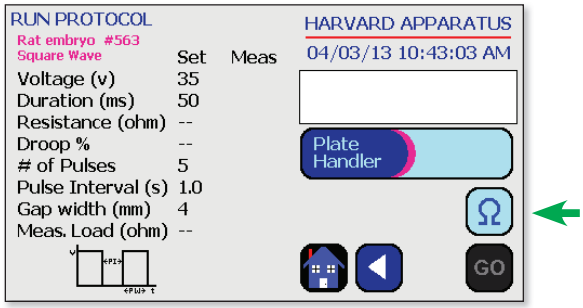
Double tap desired cells.



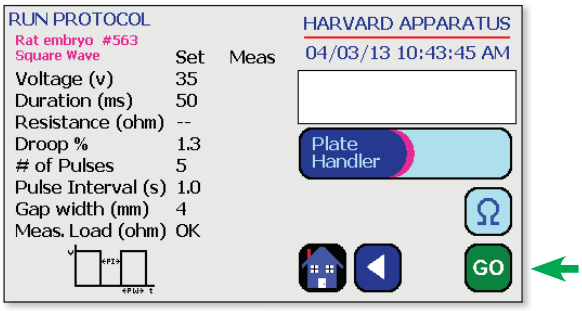
Review parameters.

Position electrodes on area being electroporated.

To run tap the Omega icon.

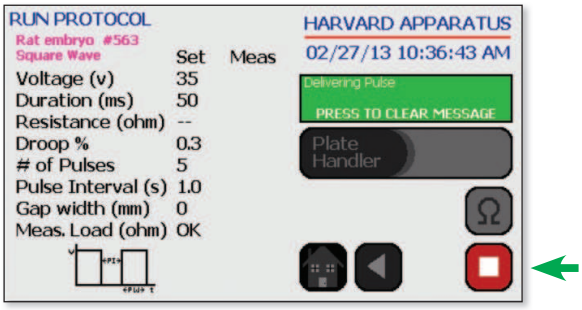


With load measurement OK, press the Go icon to run protocol.



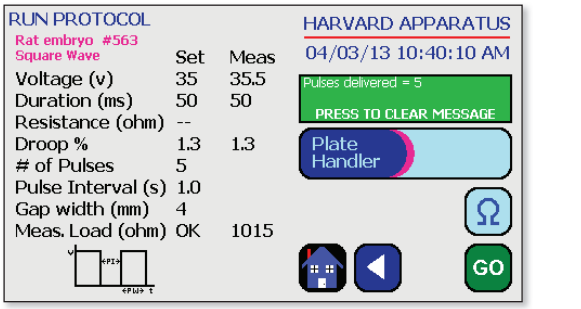
While the pulse is being delivered, the Square icon can be used to stop the protocol at any time.

NOTE: Generator will display readings after each pulse.



Once protocol is complete, data regarding your pulse is displayed and stored in the systems logs for future use.

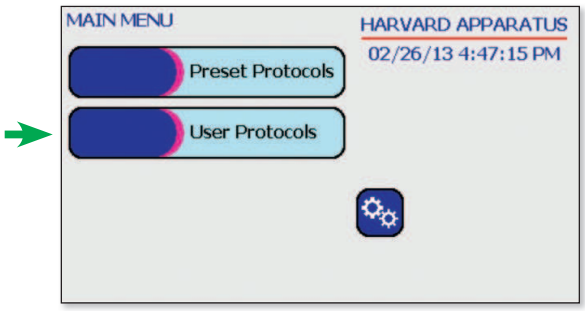
You may continue pulsing, go back to the settings screen or the protocol select screen, or to the home screen.



Deleting a Protocol

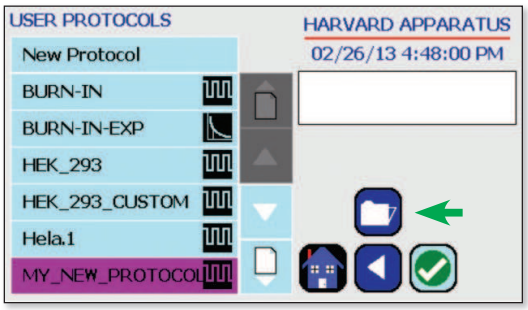
NOTE: Preset protocols cannot be deleted.

On the home screen, tap the User Protocols icon.

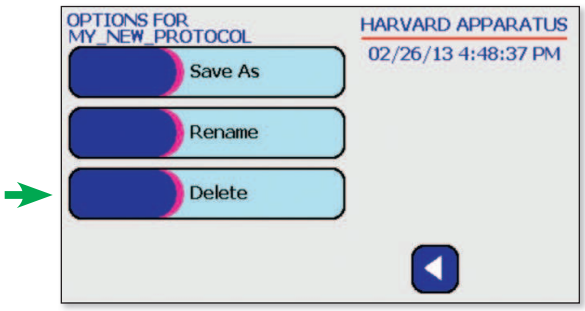


Tap the protocol you wish to delete.

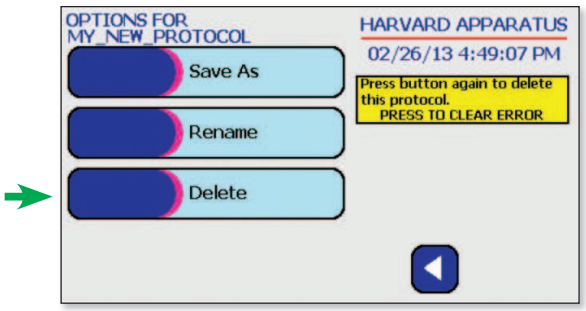
Tap Folder icon.



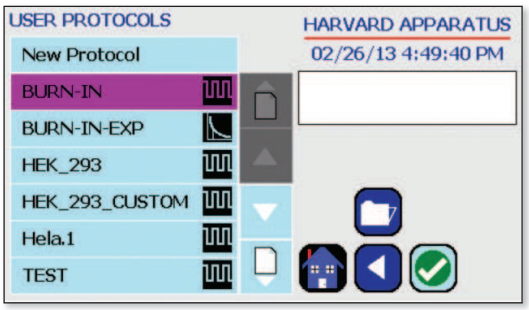
Tap Delete icon.



Confirm delete by tapping the Delete icon again.

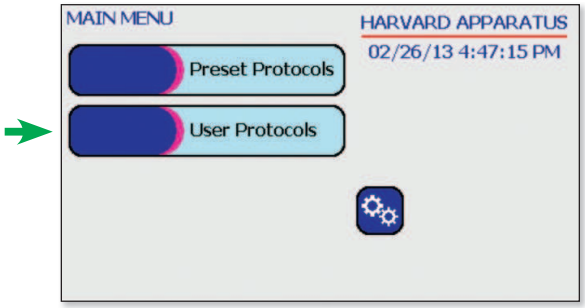


Protocol has been removed from the menu.

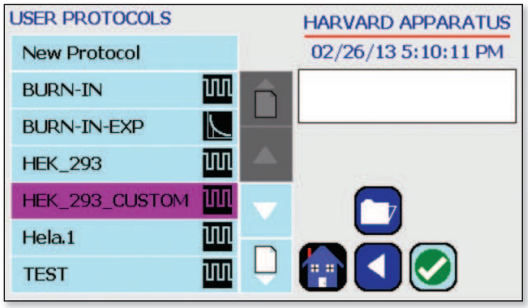


Password Protecting a Protocol

On the home screen, tap the User Protocols icon.

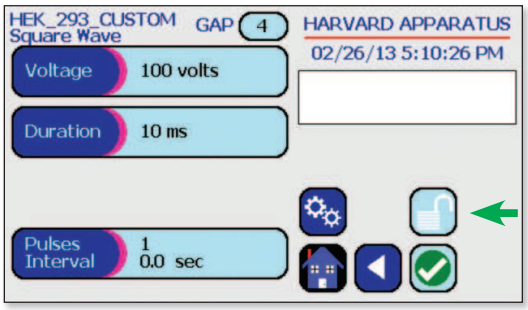


Double tap protocol you wish to password protect.

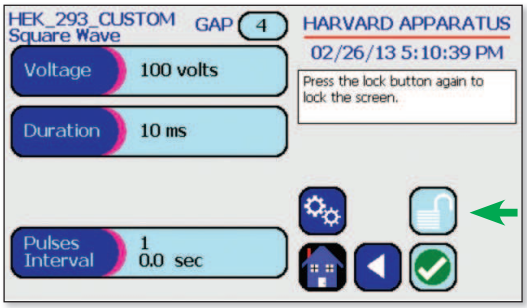


View the protocol.

Tap the Open Lock icon.

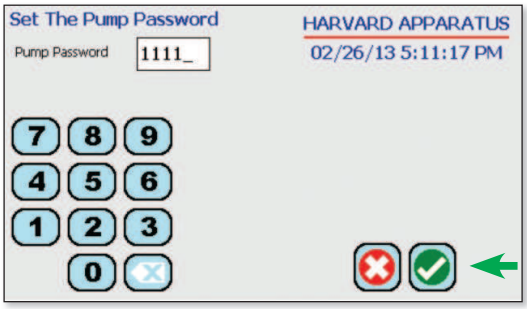


Confirm password protection by tapping the Open Lock icon again.



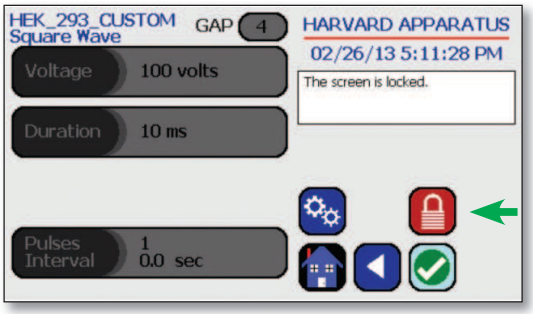
Use the numeric keypad to set the password.

Tap the Green Check icon to save.



The protocol is now locked against modifications.

To Unlock press the lock icon. Type in the password then Tap the Green Check icon.



Touchscreen Button Reference

Accept
Used to accept the parameters/settings on a screen and advance to the next screen in the menu, also used in place of a double tap on various icons.

Back
Used to go back one screen.

Cancel
Used to cancel any changes/entries on a screen and return to the previous screen in the menu.

Exponential Decay Wave Indicator
Used to indicate when a protocol utilizes exponential decay wave pulses.

File Options
Access the File Option menu that is used to save, rename and delete protocols.

Home/Main Menu
Access Preset Protocols, User Protocols and Settings.

Page Down
Used to page down in a display list.

Page Up
Used to page up in a display list.

Pre-Pulse Resistance Measurement
Used to measure the resistance of the sample prior to delivering the DC pulse.

Run Protocol
Used to deliver the pulse protocol to the sample.

Scroll Down
Used to scroll down in a display list.

Scroll Up
Used to scroll up in a display list.

Settings
Access the settings menu used to adjust the following parameters: Date and Time, Audible Alarms, Backlighting, and software updates. Displays device information.

Stop Protocol
Used during the pulse delivery sequence to stop the progress of the protocol.

Square Wave Indicator
Used to indicate when a protocol utilizes square wave pulses.

Touch Screen Locked
Indicates that the touch screen is currently locked. Press the icon and enter password to unlock the touch screen.

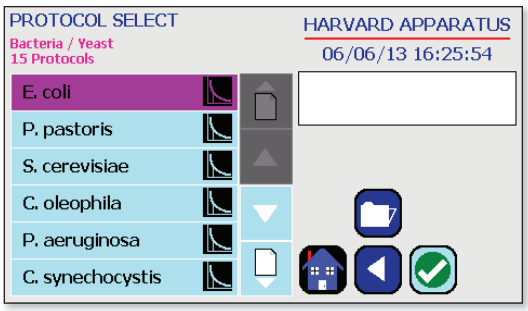
Touch Screen Unlocked
Indicates that the touch screen is currently unlocked. Pressing the icon twice will allow the user to password protect the protocol.

Error Messages & Troubleshooting

Display Error Message	Error Description	User Troubleshooting
Power Loss Error	Power loss during pulsing.	Power lost while protocol was runing.
PFC Over Temp	Pfc module over temperature.	Hardware error. Please contact the manufacturer.
Interlock Open	Electrode interlock open.	Connect electrode connectors to the front of the generator and make sure the cuvett safety dome cover is closed.
IGBT Switch Fault	IGBT switches fault.	Hardware error. Please contact the manufacturer.
Charge Voltage Max Error	Charging voltage above the expected voltage.	Turn off the unit for a few minutes then turn back on. Re-run the protocol if the issue recurs, contact the manufacturer.
Charge Current Max Error	Charging current above the expected current.	Turn off the unit for a few minutes then turn back on. Re-run the protocol if the issue recurs, contact the manufacturer.
LV Cap Bank Max	Low voltage capacitor bank voltage above expected value.	Turn off the unit for a few minutes then turn back on. Re-run the protocol if the issue recurs, contact the manufacturer.
LV Cap Bank Min	Low voltage capacitor bank voltage below expected value.	Turn off the unit for a few minutes then turn back on. Re-run the protocol if the issue recurs, contact the manufacturer.
HV Cap Bank Max	High voltage capacitor bank voltage above expected value.	Turn off the unit for a few minutes then turn back on. Re-run the protocol if the issue recurs, contact the manufacturer.
HV Cap Bank Min	High voltage capacitor bank voltage below expected value.	Turn off the unit for a few minutes then turn back on. Re-run the protocol if the issue recurs, contact the manufacturer.
Charge Time Max	Charging capacitor bank to selected voltage exceeded max time estimated.	Turn off the unit for a few minutes then turn back on. Re-run the protocol if the issue recurs, contact the manufacturer.
Pulse Droop Error	Pulse voltage droop exceeded estimated droop.	A) Increase the sample resistance. This can be achived by: 1. Increasing the gap size 2. Reducing the volume 3. Use a less conductive buffer B) Reduce the pulse duration
Pulse Voltage Overshoot	Pulse voltage monitor (p-vmon) pulse overshoot.	Hardware error. Please contact the manufacturer
Pulse Voltage Mon too Low	Pulse voltage monitor (p-vmon) below min voltage.	Hardware error. Please contact the manufacturer
Pulse Voltage Mon too High	Pulse voltage monitor (p-vmon) above max voltage.	Hardware error. Please contact the manufacturer
Pulse Current Mon too Low	Pulse current monitor (p-imon) below min current.	Hardware error. Please contact the manufacturer
Pulse Current Mon too High	Pulse current monitor (p-imon) above max current.	Hardware error. Please contact the manufacturer
Arc_Detected	Arc detected during pulsing.	Arc could happen due to the one or more of the following conditions: 1. Sample resistance is very low for the voltage selected 2. Gap size is too small for the voltage selected 3. Pulse duration is too long
No_HB_from_GUI	Communication error.	Hardware error. Please contact the manufacturer
Sample Resistance Out Of Range	The measured sample load is out of range.	Increase the sample resistance. This can be achived by 1. Increasing the gap size 2. Reducing the volume 3. Replace the buffer with less conductive type of buffer
Over Current Pulse Abort	Pulse aborted due to pulse over current.	The over current protection feature is added to prevent sample arcing. This could occur when the sample resistance changes during the pulse: 1. Sample resistance is very low for the voltage selected 2. Gap size is too small for the voltage selected 3. Pulse duration is too long
Idle V I Max	Voltage or current in idle state is higher than expected.	Turn off the unit for a few minutes then turn back on. Re-run the protocol if the issue recurs, contact the manufacturer
Charge Cap Bank A Max	Charge capacitor bank A voltage is above tolerance.	Turn off the unit for a few minutes then turn back on. Re-run the protocol if the issue recurs, contact the manufacturer
Charge Cap Bank A Min	Charge capacitor bank A voltage is below tolerance.	Turn off the unit for a few minutes then turn back on. Re-run the protocol if the issue recurs, contact the manufacturer
Charge Cap Bank B Max	Charge capacitor bank B voltage is above tolerance.	Turn off the unit for a few minutes then turn back on. Re-run the protocol if the issue recurs, contact the manufacturer
Charge Cap Bank B Min	Charge capacitor bank B voltage is below tolerance.	Turn off the unit for a few minutes then turn back on. Re-run the protocol if the issue recurs, contact the manufacturer
Charge Cap Bank C Max	Charge capacitor bank C voltage is above tolerance.	Turn off the unit for a few minutes then turn back on. Re-run the protocol if the issue recurs, contact the manufacturer
Charge Cap Bank C Min	Charge capacitor bank C voltage is below tolerance.	Turn off the unit for a few minutes then turn back on. Re-run the protocol if the issue recurs, contact the manufacturer
Dump Time Max	The capacitor bank did not fully discharge in the estimated time.	Turn off the unit for a few minutes then turn back on. Re-run the protocol if the issue recurs, contact the manufacturer
Invalid Protocol	The generator was requested to run an invalid protocol.	Verify that the used protocol is within the allowed specifications of the generator
System Processing (Busy)	The control command is invalid or was issued while the generator was busy.	Allow more time between events
TC_Error	The measured rc time while delivering an exponential decay pulse is invalid.	Hardware error. Please contact the manufacturer
Pulse Duration Error	The pulse duration did not meet the expected duration.	Hardware error. Please contact the manufacturer
Unit Type Sel Error	The unit hardware and software do not match the corect type.	Hardware error. Please contact the manufacturer
Error: 0X#	Multiple errors.	Multiple error. Please contact the manufacturer

Managing Gemini X² Series Protocols

All Preset and User-Defined Protocols are stored in the Protocol List for ongoing use. Over time, you may want to manage previously created Protocols to keep your Protocol List easy to navigate. In addition, the BTX Gemini X² allows you to export Protocol information to an externally connected generator or to a computer that will be used to control operation. This section of the Guide describes the file management facilities available on the BTX Gemini X².



Saving a Copy of a Protocol

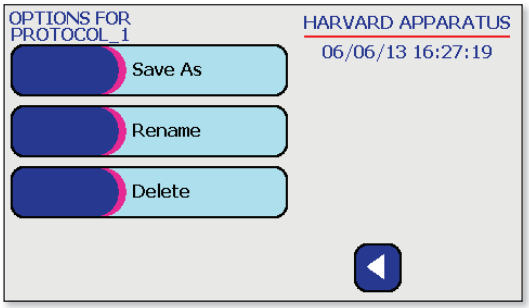
Follow these instructions to save a copy of a Protocol under a new name:

1. From **Protocol Configuration** screen, choose the **Protocol Name** button to enter the **Mode Selection** screen.
2. From the list of available Protocols, select (press once) the Protocol you want to save a copy of, then choose the **File Options** button to enter the File Options screen.
3. Choose **Save As/Copy** to access the **Protocol Name Entry** screen. The current **Protocol Name** will be displayed in the text entry box.
4. On the **Protocol Name** screen, type the new name for your Protocol using the onscreen keyboard. Choose the Symbols/ Numbers button (.?123) to display the list of available numbers and symbols that can be included in the Protocol Name. To switch the keyboard back to letters mode, choose the Letters button (ABC). [15 characters maximum]
5. When finished entering the new Protocol Name, choose **Accept** to save your changes and return to the **File Options** screen.
6. Choose **BACK** to return to the Mode Selection screen

Renaming a Protocol

Follow these instructions to rename a previously saved user protocol:

1. From the **Protocol Configuration** screen, choose the **Protocol Name** button to enter the **Protocol Mode** screen.
2. From the list of available Protocols, select (press once) the Protocol you want to rename, then choose the **File Options** button to enter the **File Options** screen.
3. Choose Rename Protocol to access the **Protocol Name Entry** screen. The current Protocol Name will be displayed in the text entry box.
4. On the **Protocol Name** screen, type the new name for your Protocol using the onscreen keyboard. Choose the Symbols/ Numbers button (.?123) to display the list of available numbers and symbols that can be included in the Protocol Name. To switch the keyboard back to letters mode, choose the Letters button (ABC). [15 characters maximum]
5. When finished entering the new Protocol Name, choose **Accept** to save your changes and return to the File Options screen.
6. Choose **Back** to return to the Protocol.



Protocol Manager

Installation

Insert the generator CD into the CDROM, a file containing the CD's contents will open automatically. Navigate to the Protol Manager folder, under Applications. Right click on "Protocol Manager". Select "Send To" then "Desktop" to create a shortcut on your desktop.

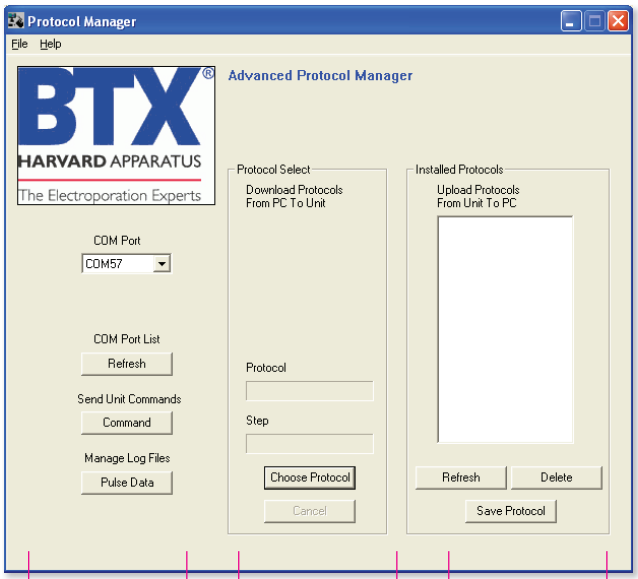
NOTE: Compatible with Windows XP & Windows 7.

1. Connect the generator to the PC using a USB cable.
2. Turn generator on.
3. Start Protocol Manager.
4. Select the proper COM port.

Overview

Protocol Manager allows the user to:

- Upload protocols from the Gemini X² generator to a PC.
- Download protocols from PC to the Gemini X² generator.
- Send commands to the Gemini X² generator using a PC.
- Upload log files from the Gemini X² generator to PC.

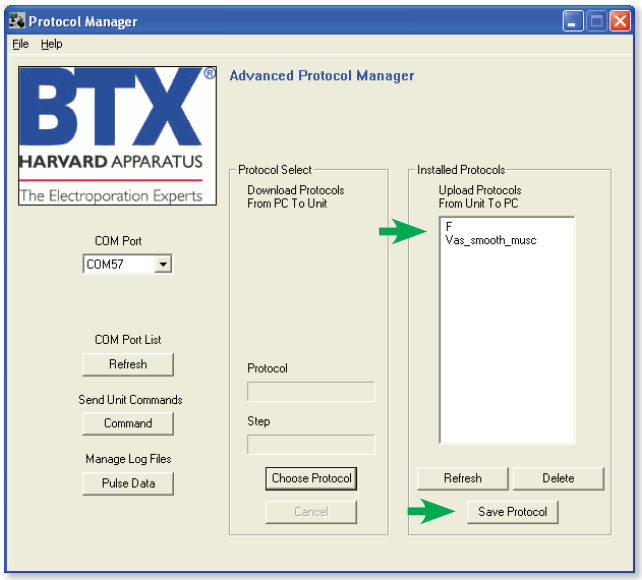


Log File Control | PC to Generator Protocol Control | Generator to PC Protocol Control

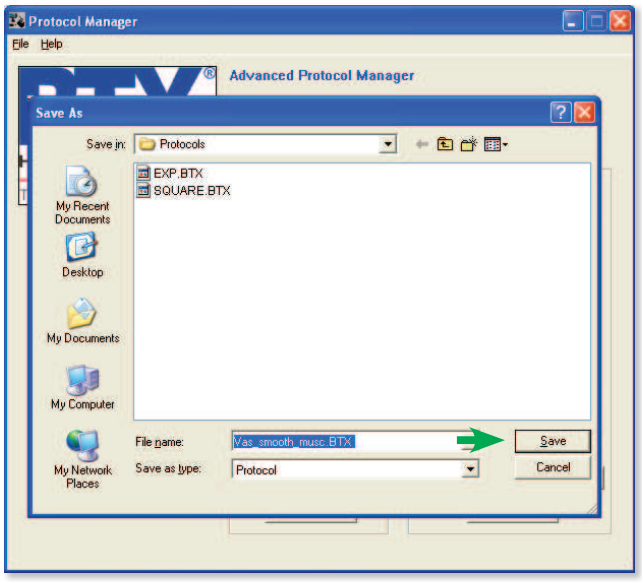
Upload– Generator to PC

1. Select Protocol(s)
2. Click 'Save Protocol'

NOTE: Hit 'Refresh' to update the list after creating or editing protocols on the Gemini X² generator.



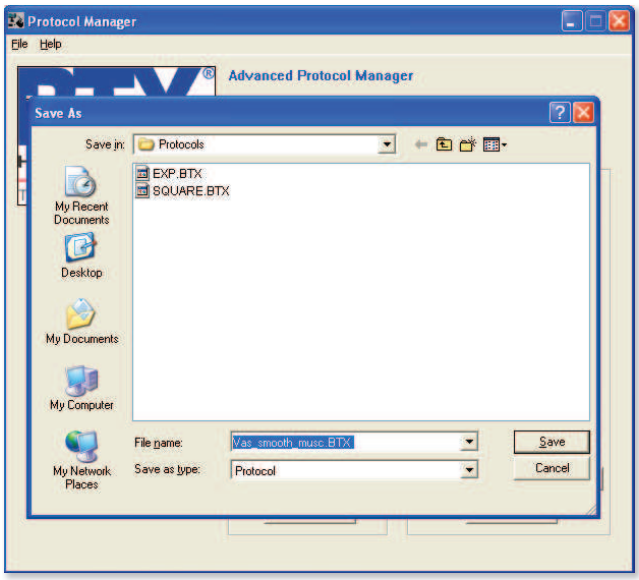
3. Select the drive and folder location
4. Click 'Save'



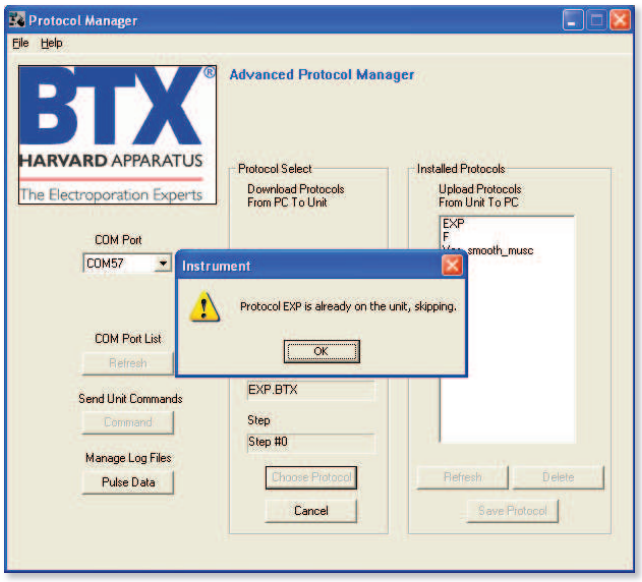
Protocol Manager

Download - PC to Generator

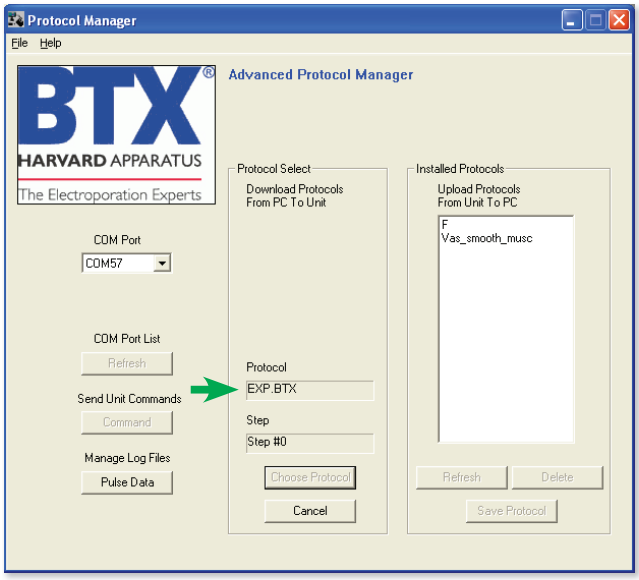
1. Click 'Choose Protocol'
2. Select Protocol(s) to download
3. Click 'Open'



NOTE: If a protocol already exists on the Gemini X² generator, Protocol Manager will not overwrite the file if you attempt to re-download it.



4. Protocol name being dowloaded is shown

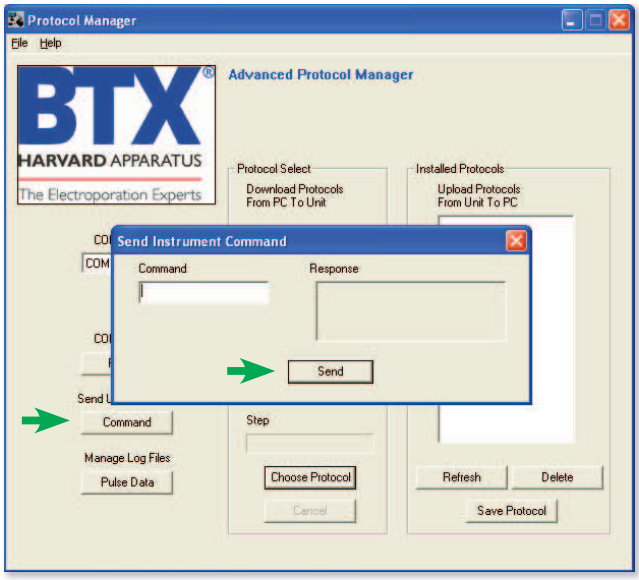


Protocol Manager

Sending Commands to Generator

1. Click 'Command'
2. Type desired command
3. Click 'Send'

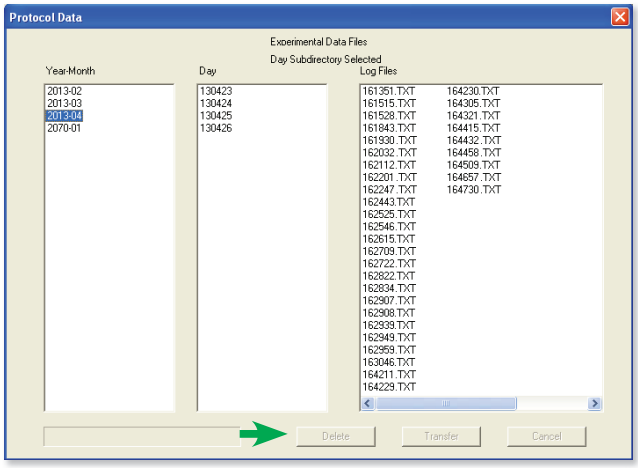
NOTE: If unexpected responses are seen, type 'echo off'.



Upload Log Files

1. On the main screen, click 'Pulse Data'
2. Select your desired files or folders and then click 'Transfer'.

NOTE: Files should be backed up periodically. The generator's SD card can store up to approximately 100,000 files.



3. Select the drive or folder location.

Troubleshooting Protocol Manager

1. Do not upload/download Protocols while generator is running. Program works best while generator is idle on Protocol setup screen.
2. Order of Operations:
   A. Connect USB.
   B. Turn generator on.
   C. Start Protocol Manager program.

Maintenance

The BTX Gemini Twin Wave Series generators require no special maintenance other than keeping them clean.

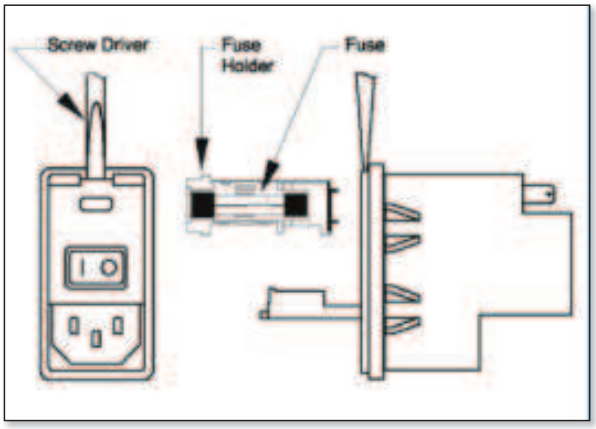
To clean the exterior surfaces, use a lint-free cloth to remove loose dust. Use care to avoid scratching the clear display window. For more efficient cleaning, use a soft cloth dampened (not soaked) with an aqueous solution of 75% isopropyl alcohol, or a mild detergent.

Fuse Replacement

Key Information

1. Make sure the power cord is disconnected from the main supply before servicing the fuse.
2. Use only Type 3AG, 1/4 x 11/4 in, 5 amp, 250 volt; Type T (time delay) fuses

Turn off power and remove power cord from power module. Use a straight blade screw-driver to pry open the access door. Remove the fuse holder and then remove the fuses from this holder as shown in figure below. Replace fuses, and then replace the fuse holder.



Battery Replacement

1. Remove screw and swing cover to the side.
2. Slide battery out from under clip.
3. Install Lithium Coin Battery: 3V, 16mm CR1620 by sliding under clip with positive side facing out

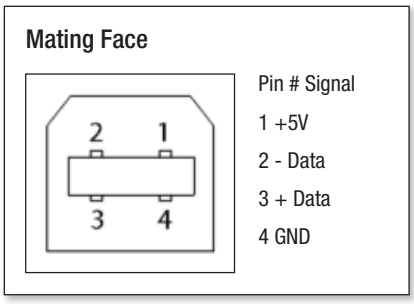
CAUTION: Use only same type and rated battery. Observe polarity when installing.

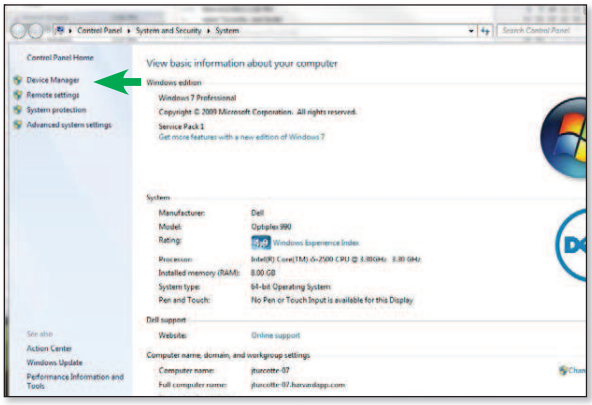
Upgrading BTX Gemini Series Software

USB Virtual Commport Driver Installation

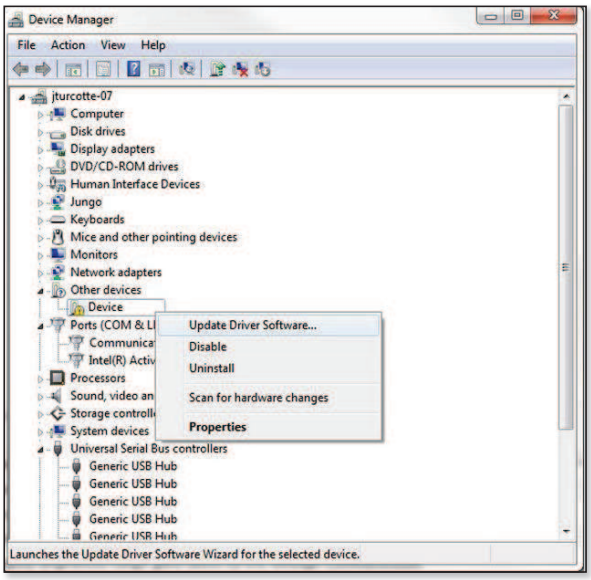
When you connect the BTX Gemini X² to a computer via USB for the first time, Windows will seek to install a driver for communication. The following section details the installation of the Virtual CommPort Driver supplied with the BTX Gemini X².

1. Connect the Gemini X² generator to the computer via USB. Open the control panel and select **System and Security** and then **System**. Click Device Manager on the left hand menu.

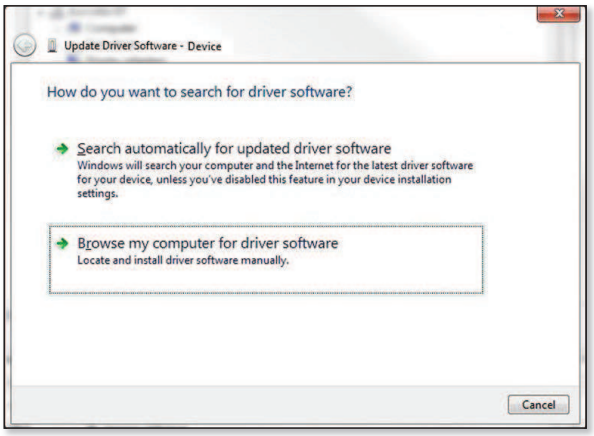




2. Two Flash update applications will be displayed in the device manager, under **Other Devices**. Right click on either of the flash update applications and select **Update Driver Software**.

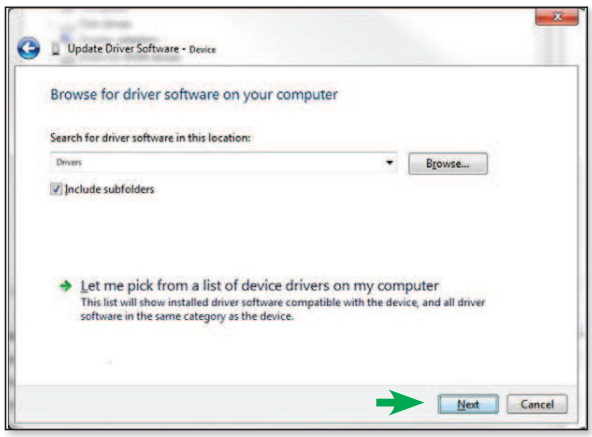


3. Select Browse my computer for driver software

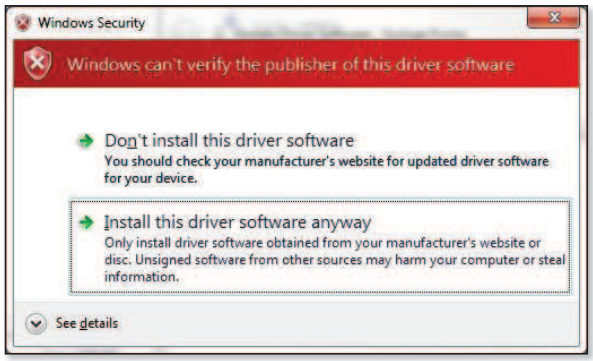

Update Driver Software - Device
How do you want to search for driver software?
Search automatically for updated driver software
Windows will search your computer and the Internet for the latest driver software for your device, unless you've disabled this feature in your device installation settings.
Browse my computer for driver software
Locate and install driver software manually.
Cancel

USB Virtual Commport Driver Installation (continued)

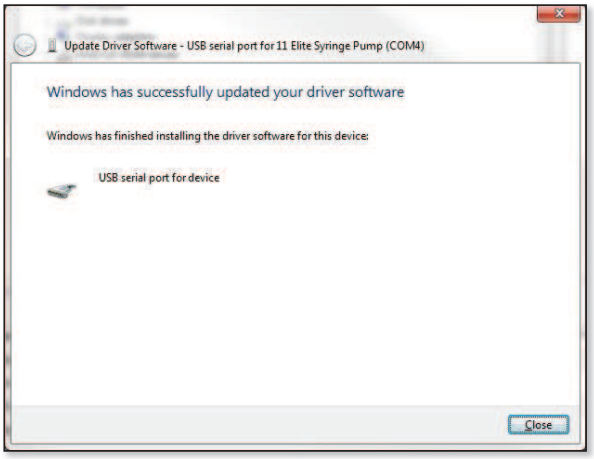
4. Click Browse and navigate to the generator drivers folder located on the CD supplied with the generator. Select the file BTX USB-CDC.inf. Make sure the **Include Subfolders** box is checked and then click **Next**.


Update Driver Software - Device
Browse for driver software on your computer
Search for driver software in this location:
Drivers
Browse...
Include subfolders
Let me pick from a list of device drivers on my computer
This list will show installed driver software compatible with the device, and all driver software in the same category as the device.
Next
Cancel

5. If a warning message regarding the driver publisher is displayed, choose **Install this driver software** anyway.


Windows Security
Windows can't verify the publisher of this driver software
Don't install this driver software
You should check your manufacturer's website for updated driver software for your device.
Install this driver software anyway
Only install driver software obtained from your manufacturer's website or disc. Unsigned software from other sources may harm your computer or steal information.
See details

6. Click **Close** after the software has been successfully installed.


Update Driver Software - USB serial port for 11 Elite Syringe Pump (COM4)
Windows has successfully updated your driver software
Windows has finished installing the driver software for this device:
USB serial port for device
Close

Upgrading BTX Gemini Series Software

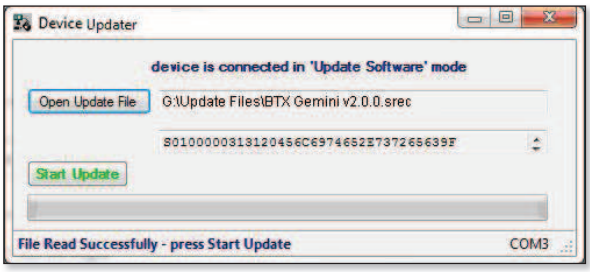
Device Updater Program

NOTE: Prior to upgrading, users will need to install USD drivers as well as the bootloader driver file. Reference 'Virtual Comport Driver Installation'.

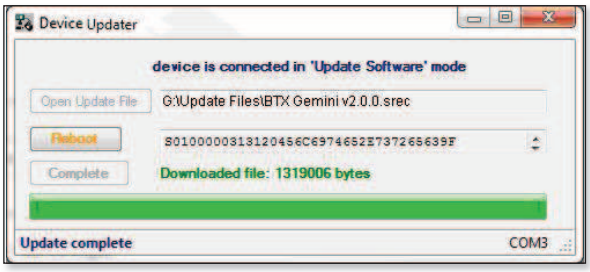
1. Upload the latest software versions to your desktop (format is filename.srec). There are two .srec files that both need to be updated: 'BTX Gemini vx.x.x' and a 'BTX Gemini Pwr vx.x.x'.
2. Disconnect all I/O devices and then connect the Gemini X^2 generator to PC using a USB cable.
3. On the Set up screen of the Gemini X^2 generator, press the **Upgrade Software** button twice to enter **Boot Loader Mode**.

NOTE: To exit boot loader mode without upgrading the software, power cycle the generator.

4. From the CD provided with the Gemini X^2 generator, open the **Device Updater Application**.



5. Click Open Update File and browse to the 'BTX Gemini vx.x.x.srec'. file that was previously saved to the desktop.
6. Click **Start Update**.



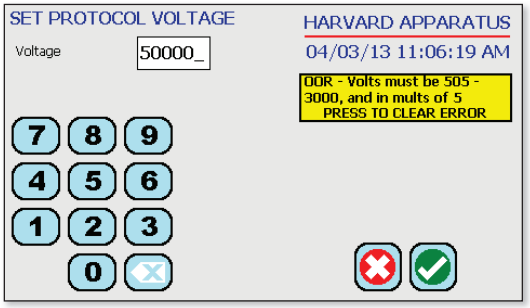
7. After the update is complete, press Reboot and then power cycle the Gemini X^2 generator from the power switch in the back of the unit.
8. Repeat steps 5 through 7 with the 'BTX Gemini Pwr vx.x.x.srec'.

Troubleshooting

NOTE: It is recommended that you save your protocols periodically by uploading them to a PC.

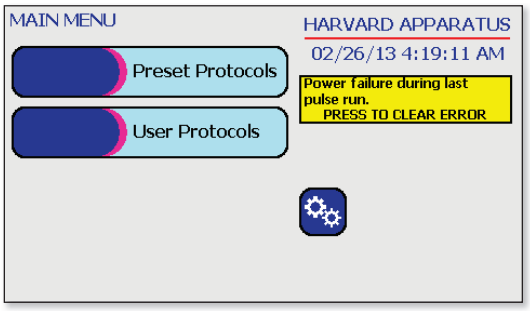
Out of Range

A value was entered or encountered in a protocol that was beyond the generator's limits.



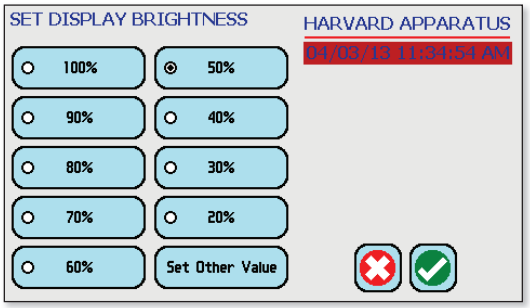
Power Failure Notification

If power is interrupted during operation, an alarm will sound to alert the user to the interruption. When power is restored, an onscreen message is displayed to indicate the interruption.



Low Battery Indication

Time/Date area will turn red when battery is low.



General Optimization Guide for Electroporation

As described, electroporation is the application of controlled direct current (DC) electrical pulses which are applied to living cells and tissues for a short duration of time. The pulse induces a transmembrane potential which causes the reversible breakdown of the cellular membrane. This action results in the permeation or "pore formation" of the cell membrane which allows small molecules (such as dye, oligonucleotides or peptides) and large molecules (such as proteins, DNA and RNA) to be introduced into the cell. During this process the cellular uptake of the molecules continue until the pores close which can take milliseconds to minutes.

Optimization of the electroporation process involves several factors. Choosing the wave form, determining field strength and adjusting pulse length are just a few critical variables. Other parameters which play a crucial role in optimization include cell diameter, plasmid concentrations, temperature and electroporation buffer.

Wave Forms

Pulse shape generally falls into two categories, square wave or exponential decay wave:

Square Wave Pulse

Square wave pulses rise quickly to a set voltage level, maintains this level during the duration of the set pulse length and quickly turns off. Square waves yields higher efficiencies and viabilities in mammalian cells. Square wave EP in **in vivo** and **ex vivo** tissues, embryo's, and plant protoplast applications yield better results in comparison to an exponential decay wave.

Exponential Decay Wave Pulse

Exponential decay waves generate an electrical pulse by allowing a capacitor to completely discharge. A pulse is discharged into a sample the voltage rises rapidly to the peak voltage set then declines over time. The powerful exponential decay wave pulse is routinely used for transformation of gram-negative and gram-positive, bacterial, yeast, plant tissues, insect cells and some mammalian cells.

Field Strength

The field strength is measured as the voltage delivered across an electrode gap and is expressed as kV/cm. Field strength is critical to surpassing the electrical potential of the cell membrane to allow the temporary reversible permeation or "pore formation" to occur in the cell membrane. Three factors should be considered for optimizing field strength:

1. Electrode Gap Size
2. Cell Diameter
3. Temperature

Cell Types Field Strength Ranges

Bacteria/Yeast: 3-24 kV/cm

Mammalian: 0.25-3 kV/cm

Plant: 3-12 kV/cm

Electrode Gap Size

The distance between electrodes, or "gap size" is important when optimizing your electroporation experiment. Field strength is calculated using voltage divided by gap size. For example, using a 4mm gap cuvette with 500V would provide a field strength of 1.25kV/cm. If instead of a 4mm gap cuvette, a 2mm gap cuvette was used, the voltage would have to be reduced by half or to 250V in order to maintain the same field strength of 1.25kV/cm. It is possible to derive the voltage needed to accomplish electroporation if the desired field strength and gap size are known. The calculation for this is Field strength (kV) multiplied by gap size (cm) equals voltage. For example, if a user was certain that a 1.25 kV/cm field strength was required in a 1mm gap cuvette the calculation would be: 1.25kV x 0.1cm= 0.125kV or 125V.

Example: A field strength of 1.25 kV/cm

4mm gap cuvette = 500V

2mm gap cuvette = 250V

1mm gap cuvette = 125V

Cell Diameter

Generally, smaller cell sizes require higher voltages while larger cell diameters require lower voltages for successful cell membrane permeation.

Temperature

The temperature at which cells are maintained during electroporation effects the efficiency of the electroporation for several reasons. The majority of mammalian cell lines are effectively electroporated at room temperature. Samples which are pulsed at high voltage or exposed to multiple pulses and long pulse durations can cause the sample to heat up. These conditions cause increased cell death and lowers the transfection efficiency. Maintaining the sample at lower temperatures can diminish the heating effects on cell viability and efficiency. Since electroporation causes the transient formation of pores, keeping the cells at a lower temperature following the pulse may allow the pores to remain open longer to allow more uptake of the exogenous molecules. Yet lower temperatures on other cell lines can be damaging and cause high cell mortality. This effect is specific to each cell line and should be considered during optimization studies. The standard pulse voltage used for cells at room temperature will need to be approximately doubled for electroporation at 4°C in order to effectively permeate the cell membrane.

General Optimization Guide for Electroporation

Pulse Length

The pulse length is the duration of time the sample is exposed to the pulse. This is measured as time in micro to milliseconds ranges. Adjusting this parameter is dependent on the pulse wave form. The pulse length in a square wave system can be inputted directly. The pulse length in an exponential decay wave system is called the "time constant" which is characterized by the rate at which the pulsed energy (e) or voltage is decayed to 1/3 the original set voltage. This time constant is modified by adjusting the resistance and capacitance (RC) values in an exponential decay wave form. Time constant calculation T=RC, where T is time and R is resistance and C is capacitance.

The pulse length works indirectly with the field strength to increase pore formation and therefore the uptake of target molecules. Generally, during optimization of parameters an increase in voltage should be followed by an incremental decrease in pulse length. When decreasing the voltage, the reverse is true. Pulse length is a key variable that works hand in hand with voltage and needs to be considered when optimizing electrical parameters to maximize the results for a given cell type.

Number of Pulses

Electroporation is typically carried out as a single pulse for most cell types. However, other cell lines may require multiple pulses to achieve maximum transfection efficiencies. Usually lower voltages are used when applying multiple pulses in order to gradually permeate the cell membranes. This allows the transfer of molecules while avoiding damage to delicate or whole tissue samples. This method of multiple pulsing is critical for maximum gene delivery without causing tissue damage to *in vivo*, *in utero* and explant tissue environments. The use of multiple pulse will require the optimization of key electrical parameters including voltage and pulse length. Typically, for *in vivo* applications the use of lower voltages between 10-100 volts with pulse lengths ranging 30-50msec provides efficient transfection. The optimal voltage, pulse length and number of pulses will vary depending on the cell type and molecule (DNA or RNA) transfected.

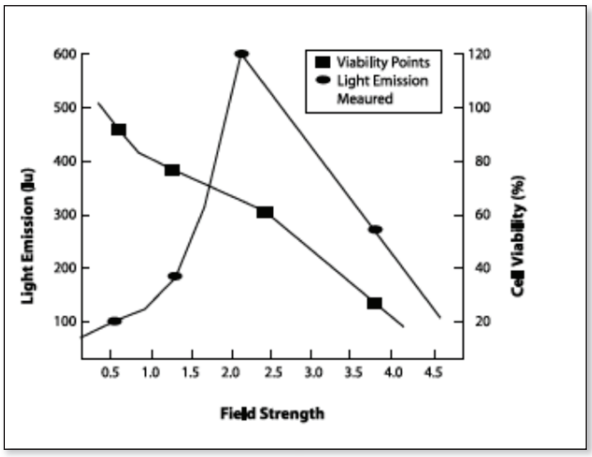
Electroporation Buffer

The buffers used for electroporation can vary depending on the cell type. Many applications use highly conductive buffers such as PBS (Phosphate Buffered Saline <30 ohms) and HBSS (Hepes Buffer <30 ohms) or standard culture media which may contain serum. Other recommended buffers are hypoosmolar buffers in which cells absorbs water shortly before pulse. This swelling of the cells results in lowering the optimal permeation voltage while ensuring the membrane is more easily permeable for many cells but can be damaging to others. Prokaryotic cells such as bacteria require the use of high resistance buffers (>3000 ohms) for this reason proper preparation and washing of the cells is essential to remove excess salt ions to reduce the chance of arcing. Ionic strength of an electroporation buffer has a direct affect on the resistance of the sample which in turn will affect the pulse length or time constant of the pulse. The volume of liquid in a cuvette has significant effect on sample resistance for ionic solutions, the resistance of the sample is inversely proportional to the volume of solution and pH. As the volumes are increased resistance decreases which increases the chance of arcing, while lowering the volume will increase the resistance and decrease the arc potential.

Harvard Apparatus BTX now offers BTXpress High Performance Electroporation Solution, a low conductance buffer that achieves higher transfection efficiencies with minimal cell toxicity. The BTXpress buffer is a single buffer developed to facilitate high efficiency gene delivery into mammalian cells.

DNA/RNA Concentrations

Electroporation is typically thought of as a nucleic acid (DNA, mRNA, siRNA and miRNA) transfer method into prokaryotic and eukaryotic cells. Electroporation is not limited to just nucleic acid delivery, it can introduce proteins, antibodies, small molecules and fluorescent dyes. The standard range of DNA used for transfections is 5-20g/ml for most cell types; however in some instances increasing the DNA concentration as high as 50g/ml improves transfection efficiency without changing other parameters. Determining the optimal DNA concentration through a DNA titration can be beneficial. The size of a molecule will have an effect on the electrical parameters used to transfect the cell. Smaller molecules (siRNA or miRNA) may need higher voltage with microsecond pulse lengths and larger molecules (DNA) may need lower voltages with longer pulse lengths. Buffers such as EDTA or Tris can drastically reduce the transfection efficiency. Therefore, we recommend resuspending DNA in distilled water. Finally, electroporating ligation mixtures into E.coli can cause arcing and reduced transformations. Diluting the ligation mixture a minimum of 1:5 with diH2O, dialysis, or ethanol precipitation can significantly improve transformation efficiencies and reduce the potential for arcing.



Applications

Mammalian Cell Transfection

Electroporation is a highly flexible technique used to genetically modify mammalian cells. Whether you are studying up or down regulation of genes, specific protein expression. This method is non-toxic and requires no expensive reagents to successfully transfect your cells. Primary cells, stem cells or established cell lines can be electroporated with yield high transfection efficiencies and great cell survival rates.

In Vivo, In Utero, In Ovo

Square waves system allow researchers to set the pulse lengths and number of pulses which is critical to ensure viable cells and tissues while still maintaining efficient transfection both *in vivo* and *ex vivo*. Electroporation mediated gene and drug delivery has been shown to substantially increase intracellular uptake and expression of DNA, siRNA and miRNA in muscle, skin, liver, kidney, testis, retina, tumors, etc. In vivo electroporation has successfully been used in embryo applications, *in utero* and *in ovo* applications in addition to transfection of Zebra fish.

Bacteria and Yeast Transformation

Electroporation has long been recognized as the most efficient means of transforming both gram negative and gram positive bacteria and yeast. Gramnegative bacteria such as coli or Helicobacter pylori are generally easier to transform than grampositive bacteria (e.g. Streptococcus pneumoniae) due to their cell wall composition. Transformation efficiencies of $1x10^{10}$ transformants/µg DNA are commonly seen for gram-negative bacteria, while for gram-positive bacteria, generally $1x10^{6}$ transformants/µg DNA are achievable.

Plant and Insect Transfection

Electroporation of plant tissue can be used to generate transgenic crops that are useful in agricultural/horticultural applications.

Insect models are also widely used throughout the scientific community to study development and gene regulation and function. The ability to introduce genes or molecules is essential to researchers working with either of these two species. This is why researchers consistently turn to Harvard Apparatus BTX for all of their electroporation needs.

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Electroporation Buffers

There are many kinds of buffers and medium used for electroporating cells. Typically, we recommend using medias without serum or antibiotics.

The following is a list of the most commonly used buffers/medium:

BTXpress – is a single buffer solution, developed to quickly and efficiently deliver genes into mammalian cells that were previously considered "hard to transfect" by chemical and other non-viral methods. This solution, in combination with BTX electroporators, provides researchers with the versatility needed for success across a broad range of cell types while maintaining critical cell viability. Transfection using this high performance electroporation solution is equally effective in delivering DNA as well as siRNA into mammalian cells.

PBS – is a buffer solution commonly used in biological research. It is a water-based salt solution containing sodium chloride, sodium phosphate, and, in some formulations, potassium chloride and potassium phosphate. The buffer's phosphate groups help to maintain a constant pH. The osmolarity and ion concentrations of the solution usually match those of the human body (isotonic).

HEPES – is widely used in cell culture, largely because it is better at maintaining physiological pH despite changes in carbon dioxide concentration (produced by cellular respiration) when compared to bicarbonate buffers, which are also commonly used in cell culture.

RPMI – is a form of medium used in cell culture and tissue culture. It has traditionally been used for growth of Human lymphoid cells. This medium contains a great deal of phosphate and is formulated for use in a 5% carbon dioxide atmosphere.

Opti-MEM – is an improved Minimal Essential Medium (MEM) that allows for a reduction of Fetal Bovine Serum supplementation by at least 50% with no change to growth rate or morphology. Opti-MEMt can be used with a variety of suspension and adherent mammalian cells, including Sp2, AE-1, CHO, BHK-21, HEK, and primary fibroblasts.

MEM Eagle – is suitable for a diverse spectrum of mammalian cell types. Various formulations available with either Hank's or Earle's salts.

DMEM – MEM is used in a wide range of mammalian cell culture applications. The high glucose version is well suited to high density suspension culture. The low glucose formula is used for adherent dependent cells.

CytoMix – is a composition of cytokines for the highly efficient and reproducible expansion of human multipotent mesenchymal stromal cells (MSCs).

Water & 10% glycerol – Typically used for bacteria

Cytoporation Media T – is a buffer designed for larger volume cell electroporation as it incorporates a low conductivity of 0.08 S/cm to reduce heating of solution during electroporation.

Cytoporation Media T4 – is a buffer designed for larger volume cell electroporation as it incorporates a low conductivity of 3.45 mS/cm to reduce heating of solution during electroporation.

Experiment Troubleshooting

Arcing

Verify electrical component functionality. Verify properties of cell sample (do cells need to be washed? Is the buffer appropriate for application?). Verify properties of transfectant molecule (Is the DNA well purified?) Try reducing the voltage or increase sample volume until arcing is no longer a problem.

Low (or no) Transfection Efficiency, or Incorporation

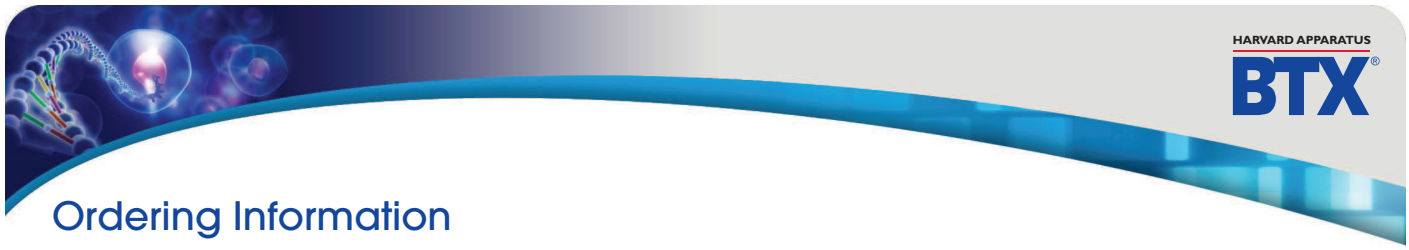
(Verify physical, biological, chemical parameters) Verify delivery of the pulse and pulse parameters. Is the voltage correct? Chamber gap? Pulse length or appropriate instrument settings? Number of pulses? If so, follow Optimization Guidelines outlined.

Low Viability

Verify physical, biological, chemical parameters. Is the voltage, chamber gap, pulse length (time constant), pulse number and other instrument settings correct? If so, reduce voltage, pulse length, or number of pulses and re-optimize protocol to improve viability as outlined .

Voltage Drop

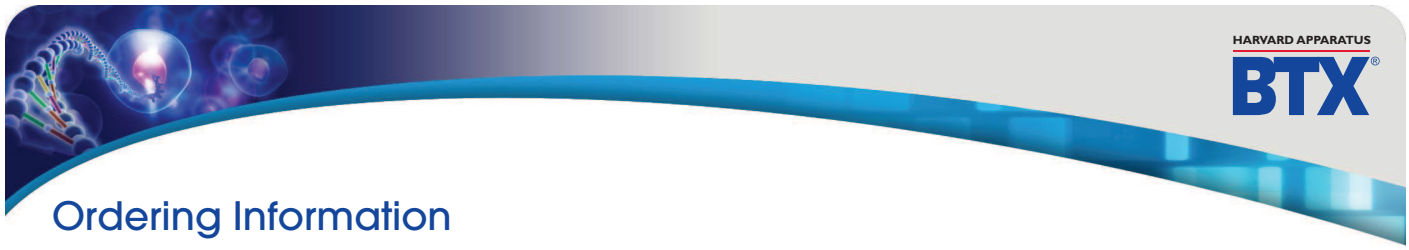
A drop in output voltage accompanies pulse delivery into highly conductive samples (for example, PBS). Thus the displayed voltage may in these situations be less than that expected, given 5% full scale accuracy and the monitoring accuracy of 5%.



Ordering Information

Order No.	Description	UOM
Electroporation Systems		
45-2001	BTX Gemini SC² Generator only	1 ea
45-2002	BTX Gemini SC² Electroporation System. Includes Gemini SC² Generator, Cuvettes - 610, 620, 640 pack of 30 (10 ea), Safety Dome 1, and 660 Cuvette Rack	1 ea
45-2006	BTX Gemini X² Generator only	1 ea
45-2007	BTX Gemini X² Electroporation System. Includes Gemini X² Generator, Cuvettes - 610, 620, 640 pack of 30 (10 ea), Safety Dome 2, and 660 Cuvette Rack	1 ea
45-2008	BTX Gemini X² HT Electroporation System. Includes Gemini X² Generator, Cuvettes - 610, 620, 640 pack of 30 (10 ea), Safety Dome 2, HT 200 Plate Handler, 1 x 2 mm gap HT Plate, 1 x 4 mm HT Plate and 660 Cuvette Rack	1 ea
Cuvettes		
45-0124	Cuvette Plus, 1 mm gap, 90 µl, pk/50, Gray	50 pk
45-0125	Cuvette Plus, 2 mm gap, 400 µl, pk/50, Blue	50 pk
45-0126	Cuvette Plus, 4 mm gap, 800 µl, pk/50, Yellow	50 pk
45-0140	Bulk Cuvette, 1 mm, 24 pk/100, Gray Case	2400 ea
45-0141	Bulk Cuvette, 2 mm gap, 24 pk/100, Blue Case	2400 ea
45-0142	Bulk Cuvette, 4 mm gap, 24 pk/100, Yellow Case	2400 ea
HT Plates (Gemin X² use only)		
45-0462	25 Well Plate, 4 mm gap, 250 µl	1 ea
45-0463	25 Well Plate, 4 mm gap, 250 µl, pk/6	6 pk
45-0466	25 Well Plate, 2 mm gap, 125 µl	1 ea
45-0467	25 Well Plate, 2 mm gap, 125 µl, pk/6	6 pk
45-0450	96 Well Plate, 2 mm gap, 125 µl	1 ea
45-0452	96 Well Plate, 4 mm gap, 250 µl	1 ea
Reagents		
45-0802	BTXpress, 5 ml	1 ea
45-0803	BTXpress, 5 ml with 2 mm gap, Cuvettes pk/50	1 ea
45-0804	BTXpress, 5 ml with 4 mm gap, Cuvettes pk/20	1 ea
45-0805	BTXpress, 10 ml	1 ea
45-0806	BTXpress, 10 ml with 2 mm gap, Cuvettes pk/50	1 ea
45-0807	BTXpress, 10 ml with 4 mm gap, Cuvettes pk/40	1 ea
47-0002	Cytoporation Media T 500 ml	1 ea
47-0003	Cytoporation Media T4 500 ml	1 ea

Order No.	Description	UOM
Accessories		
45-0400	HT 100 Plate Handler Manual	1 ea
45-0401	HT 200 Plate Handler Auto	1 ea
45-2020	BTX Safety Dome for SC²	1 ea
45-2021	BTX Safety Dome for X²	1 ea
45-2030	BTX Gemini X² Footswitch	1 ea
45-0208	Cuvette Rack	1 ea
45-0465	HT 25 Well Adapter Plate	1 ea
45-0468	Plate Handler Pins, pk/25	25 ea
45-0469	Plate Handler Pins, pk/100	100 ea
5012-017	Pliers for Plate Handler Removing Pins	1 ea
4500-012	25 Well Plate Seal	2 ea
4500-015	96 Well Plate Seal	2 ea
Cables		
45-0216	Connection Cable Cable, 10 ft, Banana to Micrograbber	1 ea
45-0204	Tweezertrode Cable / Single Adaptor Cable for Tissue Slice Electrode Positive	1 ea
45-0503	Mini Micro Grabber Adapter Cables for Tissue Slice Chamber / L Shaped Needle Electrodes	1 ea
45-0087	Micrograbber to Banana Adapter Set 45-0217	1 pr
45-2031	BTX USB Cable, 2 m	1 ea
45-2032	BTX USB Cable, 5 m	1 ea
45-0217	Electrode Cable for Flat Electrode, 10 ft BTB	1 ea
45-0088	Banana Splice F/F Adapter Set, 45-0216/45-0217	1 pr
45-0090	Adapter Set Banana to Pin Tip	1 ea
45-0089	Banana to Square Post Adapter Set for 45-0217	1 pr



Ordering Information

Order No.	Description	UOM
45-0101	Caliper Electrode, 1.0 x 1.0 cm Kit	1 Ea
45-0102	Caliper Electrode, 2.0 x 2.0 cm, 1.5 x 1.5 cm Kit	1 Ea
45-0103	Microslide 450, 0.5 mm gap, 20 µl, pk/10	10 Pk
45-0104	Microslide 450-1, 1 mm gap, 40 µl, pk/10	10 Pk
45-0105	Microslide 453, 3.2 mm gap, 650 µl	1 Ea
45-0106	Microslide 453-10, 10 mm gap, 2.0 ml	1 Ea
45-0107	Meander Fusion Chamber, 0.2 mm gap, pk/4	4 Pk
45-0108	Flat Electrode/Divergent Field, 1 mm (needs 45-0217)	1 Ea
45-0217	Electrode Cable for Flat Electrode, 10 ft BTB	1 Ea
45-0109	Flatpack Chambers, 1.83 mm gap, pk/50	50 Pk
45-0110	Flatpack Chambers, 0.56 mm gap, pk/50	50 Pk
45-0113	Genetrodes Straight, 5 mm, Gold Tip	1 Ea
45-0160	Genetrodes Straight, 5 mm, Gold Tip Kit	1 Ea
45-0114	Genetrodes Straight, 10 mm, Gold Tip	1 Ea
45-0161	Genetrodes Straight, 10 mm, Gold Tip Kit	1 Ea
45-0115	Genetrodes L-Shape, 5 mm, Gold Tip	1 Ea
45-0162	Genetrodes L-Shape, 5 mm, Gold Tip Kit	1 Ea
45-0116	Genetrodes L-Shape, 3 mm, Gold Tip	1 Ea
45-0163	Genetrodes L-Shape, 3 mm, Gold Tip Kit	1 Ea
45-0117	Genetrodes L-Shape, 1 mm, Gold Tip	1 Ea
45-0164	Genetrodes L-Shape, 1 mm, Gold Tip Kit	1 Ea
45-0203	Genetrodes/Genepaddle Holder with Shaft	1 Ea
45-0216	Genetrodes/Genepaddle Cable, 10 ft, BTMG	1 Ea
45-0122	Genepaddles, 3 x 5 mm	1 Ea
45-0169	Genepaddle, 3 x 5 mm Kit	1 Ea
45-0123	Genepaddles, 5 x 7 mm	1 Ea
45-0170	Genepaddle, 5 x 7 mm Kit	1 Ea
45-0167	2-Needle Array, 10 mm Kit	1 Ea
45-0205	2-Needle Array Handle, 10 mm (needs 45-0120)	1 Ea
45-0120	2-Needle Array, 10 mm, pk/6 (needs 45-0205)	1 Ea
45-0168	2-Needle Array, 5 mm Kit	1 Ea
45-0206	2-Needle Array Handle, 5 mm (needs 45-0121)	1 Ea
45-0121	2-Needle Array, 5 mm pk/6 (needs 45-0206)	1 Ea
45-0510	Needle L-Shaped Pt Electrode, 3 mm Kit	1 Ea
45-0509	Needle L-Shaped Pt Electrode, 3 mm (needs 45-0508)	1 Ea
45-0513	Petri, 7 mm, Tissue Chamber Kit	1 Ea
45-0505	Petri Dish Tissue Chamber, 5 x 5 mm Kit	1 Ea
45-0504	Petri Dish Tissue Chamber, 5 x 5 mm (needs 45-0216)	1 Ea
45-0506	Petri Dish Tissue Chamber, 15 x 15 mm (needs 45-0216)	1 Ea
45-0507	Petri Dish Tissue Chamber, 15 x 15 mm Kit	1 Ea
45-0100	Petri Dish Electrode, 2 mm gap, 90 mm Well Dish Kit	1 Ea
45-0130	Petri Pulser for 6 Well Plates, 35 mm Well Kit	1 Ea
45-0490	Tissue Slice Chamber, 7 x 7 mm Kit	1 Ea

Order No.	Description	UOM
45-0491	Tissue Slice Chamber, 7 x 7 mm Dish (needs 45-0492, 45-0503, 45-0204)	1 Ea
45-0492	Tissue Slice Wand, (+) 7 mm (needs 45-0491, 45-0503, 45-0204)	1 Ea
45-0500	Tissue Slice Chamber, 10 x 10 mm Kit	1 Ea
45-0501	Tissue Slice Chamber, 10 x 10 mm Dish (needs 45-0502, 45-0503, 45-0204)	1 Ea
45-0502	Tissue Slice Wand, (+) 10 mm (needs 45-0501, 45-0503, 45-0204)	1 Ea
45-0503	Mini Micro Grabber Adapter Cables for Tissue Slice Chamber / L Shaped Needle Electrodes	1 Ea
45-0530	Adherent Cell Electrode, 3 mm gap (needs 45-0204)	1 Ea
45-0531	Adherent Cell Electrode, 3 mm Kit	1 Ea
45-0486	PT Tweezertrodes, 1 mm Diameter Kit	1 Ea
45-0487	PT Tweezertrodes, 3 mm Diameter Kit	1 Ea
45-0489	PT Tweezertrodes, 5 mm Diameter Kit	1 Ea
45-0488	PT Tweezertrodes, 7 mm Diameter Kit	1 Ea
45-0165	SS Tweezertrode, 7 mm Diameter Kit	1 Ea
45-0118	SS Tweezertrode, 7 mm Diameter (needs 45-0204)	1 Ea
45-0166	SS Tweezertrode, 10 mm Diameter Kit	1 Ea
45-0119	SS Tweezertrode, 10 mm Diameter (needs 45-0204)	1 Ea
45-0524	PT Tweezertrode, 1 mm Flat (needs 45-0204)	1 Ea
45-0525	PT Tweezertrode, 1 mm Flat Kit	1 Ea
45-0204	Tweezertrode Cable / Single Adaptor Cable for Tissue Slice Electrode Positive	1 Ea
Specialty Electrodes (AgilePulse Systems Only)		
47-0090	Electrode Adapter Box for Agile Pulse *In Vivo*	1 Ea
47-0000	Parallel-Needle Array Handle for AP *In Vivo*	1 Ea
47-0040	4-Needle Array, 4 mm gap, 2 mm Length, AP *In Vivo* (ID*)	1 Ea
47-0043	3-Needle Array, 4 mm gap, 3 mm Length, AP *In Vivo* (IM)	1 Ea
47-0045	4-Needle Array, 4 mm gap, 5 mm Length, AP *In Vivo* (IM*)	1 Ea
47-0050	6-Needle Array, 4 mm gap, 2 mm Length, AP *In Vivo* (ID*)	1 Ea
47-0060	6-Needle Array, 6 mm gap, 2 mm Length, AP *In Vivo* (ID)	1 Ea
47-0070	6-Needle Array, 6 mm gap, 10 mm Length, AP *In Vivo* (IM*)	1 Ea
47-0080	6-Needle Array, 6 mm gap, 12 mm Length, AP *In Vivo* (IM)	1 Ea
47-0086	6-Needle Array, 6 mm gap, 16 mm Length, AP *In Vivo* (IM)	1 Ea
47-0204N	5 ml Chamber for Agile Pulse Max	1 Ea
47-0090	Electrode Adapter Box for Agile Pulse *In Vivo*	1 Ea
47-0206	10 ml Chamber for Agile Pulse Max	1 Ea

Declaration of Conformity

Manufacturer: *Harvard Apparatus, Inc.*
84 October Hill Road
Holliston, Massachusetts 01746-1388, U.S.A.
Phone: (508) 893-8999

We herewith declare that the following product:

Product Name: *Gemini X² Gemini SC²*

To which this declaration relates, is in conformity with the applicable EC Directives, harmonized standards, and other normative requirements:

Application of Council Directive(s): *2006/95/EC Low Voltage Directive*
2004/108/EC Electromagnetic Compatibility Directive
RoHS Compliant

Standard(s) to which conformity is declared:

Safety: *EN 61010-1:2012 (3rd Edition)*

Emmissions/Immunity:
EN 61326:2006/05/01
FR47 FCC Part I5 Sub Part B (2013)
EN 61000-4-2:2001
EN 61000-4-3:2002
EN 61000-4-4:2004
EN 61000-4-5:2001
EN 61000-4-6:2003
EN 61000-4-8 1993+A1:2000
EN 61000-4-11:2004
EN 61000-3-2:2000
EN 61000-3-3:2002
CISPR11:2003+A1:2013, Class A

EMC and Safety compliance were evaluated by Intertek/ETL Semko

Reference test report file numbers: *101111687 BOX-001, -002, -003, -004, -005*

I, the undersigned, hereby declare that the equipment specified above conforms to the above Directive(s) and Standard(s).

Place: *United States of America*
Date: *April 23, 2013*

CE

HARVARD APPARATUS

(Signature)

Beth Bauman
(Full Name)

VP Engineering / Operations
(Position)